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VIRUS DISEASES OF SWEET POTATO IN EAST AFRICA. II. TRANSMISSION TO ALTERNATIVE HOSTS [1]

F. M. L. Sheffield

SUMMARY

Virus A of sweet potato was transmitted by grafting, but not mechanically, to some species of *Ipomoea*. It was not transmitted to some solanaceous plants by means of aphids. Virus B, the more important of the 2 viruses in East Africa, was not transmitted mechanically to sweet potato but was so transmitted to some other hosts. Plants of several species of *Ipomoea* and of some members of the Solanaceae became infected with virus B when inoculated mechanically, by graft, and/or by means of white flies. All such plants showed systemic symptoms that varied in intensity among the species. In *Gomphrena globosa L.*, the virus was confined to local lesions. All attempts to transmit virus B to members of the Chenopodiaceae, Compositae, Euphorbiaceae, and Malvaceae failed.

Both viruses seem to be distinct from others described in sweet potato; virus B could not be identified with any of the white-fly-transmitted viruses previously recorded.

Two viruses that affect sweet potato, *Ipomoea batatas* (L.) Lam., in East Africa have already been described (5). Virus A, transmitted by *Myzus persicae* (Sulz.) caused no symptoms in the variety in which it was found and only mild diseases in other varieties. Virus B, transmitted by *Bemisia tabaci* (Genn.), exists in several strains some of which cause very severe diseases. The former virus was found in 1 locality only but the latter was widespread. In the search for alternative hosts, work was therefore mainly concentrated on virus B. The plants tested included hosts of other viruses known to be transmitted by white flies, common weeds, and some of those species usually used as virus test plants.

MATERIAL AND METHODS.—Whenever possible, the test plants used were young seedlings, but when a variety was heterozygous (e.g. cassava) it was necessary to use small-sized rooted cuttings. Several experiments with each host were usually done at the same time; controls, numbering about half the total of experimental plants, were set up. As none became infected, they will not usually be referred to.

In the earlier experiments, species of *Ipomoea* were the test plants, and the suspected plants were tested back to sweet potato by grafting or by insect transmission before any results were recorded as positive. In the later experiments with other genera, the suspected plants were tested by mechanical transmission to a cultivated species of *Ipomoea* that is near *Ipomoea purpurea* L. This species was chosen because it becomes infected more readily following mechanical inoculation than do some others, it shows unmistakable symptoms (Fig. 1) relatively quickly, and it produces much viable seed that germinates rapidly. This species will be called the "Ipomoea test species."

Insect transmission.—The methods usually used were those that had been found best in transmitting the viruses to sweet potato varieties (5). For experiments with virus A, aphids were starved overnight before being fed for 2 minutes on infected sweet potato and then transferred to the plant species under test. For virus B, white flies were reared on infected sweet potatoes and were then transferred to the test plants. (White flies had been found to be inefficient vectors under experimental conditions, and it seemed possible that they might acquire the virus more readily from hosts other than sweet potato. When the insects were reared on an infected plant of the Ipomoea test species, however, and then transferred 50 at a time to sweet potato cuttings, only 6 of 21 plants and 1 of 6 plants became infected in 2 experiments.)

Graft transmission.—When a species was sufficiently closely related to a known host for there to be a good chance of a successful union taking place, top cleft grafts with infected scions were made.

Mechanical transmission.—It had not been found possible to transmit either virus to sweet potatoes by mechanical inoculation with juice extracted from sweet potatoes (5). The first alternative host found in the insect transmission experiments was the species of *Ipomoea* later used as the test species, and from this was started a series of experiments with mechanical methods. Juice was extracted from infected plants into water and into 0.5 per cent sodium sulphite. These juices were rubbed over leaves of 3 young seedlings of the same species, and the experiments were repeated with Celite added to the juices. All 6 of the plants rubbed with water extracts became infected; symptoms appeared in about 15 days. Of the plants rubbed with sulphite extracts, only 4 became infected, and symptoms did not appear until 3 weeks after inoculation. In all further experiments, an aqueous extract and Celite were used. It was seldom that all inoculated plants became infected; hence very little water was used, and the leaves were rubbed with as little delay as possible after the extract was made. Leaves of controls were rubbed with Celite and water.

RESULTS.—*Mechanical transmission of virus obtained from several sources.*—Whereas virus B was transmitted mechanically to 2 different plant species, even when the inoculum was taken from sweet potato (Table 1), it was not possible to transmit it mechanically to sweet potato by the methods used, whatever the source of the inoculum. None of the plants tested became infected with virus A following mechanical inoculation.

[1] Accepted for publication June 27, 1957.

Comparison of virus strains.—When compared on the same sweet potato varieties, viruses obtained from 15 naturally infected specimens fell into 6 groups, 5 of which were strains of virus B (5). Four plants

FIG. 1–5. FIG. 1. Leaves of Ipomoea test species. Left to right: Infected with virus A, healthy, and infected with virus B ($\times\frac{1}{2}$).—FIG. 2. Leaf of *Lycopersicum esculentum* infected with virus B ($\times\frac{2}{3}$).—FIG. 3. Leaf of *Datura ferox* infected with virus B ($\times\frac{2}{3}$).—FIG. 4. Left: Healthy plant of *Nicotiana glutinosa*. Right: Plant of same species infected with virus B ($\times\frac{1}{10}$).—FIG. 5. Colour breaking in flower of *Petunia* sp. infected with virus B ($\times\frac{2}{3}$).

TABLE 1.—*Mechanical transmission of sweet potato viruses obtained from different sources*

Source of inoculum	Virus	No. of each new host infected[a]		
		Ipomoea test species	*I. batatas* var. Muguga C	*Petunia* sp.
Ipomoea batatas var. Muguga C[b]	A	0/3, 0/3, 0/3	0/3	0/3
Ipomoea test sp.[c]	A	0/3, 0/3	0/3, 0/3	
I. batatas[d]	B	1/3	0/3	2/3
I. batatas var. Muguga C[b]	B	3/3	0/3	0/3
Ipomoea test sp.[e]	B	3/3	0/3	1/3
Ipomoea test sp.[b]	B	2/3	0/3	1/3
Ipomoea test sp.[e]	B	1/3	0/3	1/3
Petunia sp.[b]	B	3/3	0/3	1/3

[a] Denominator shows number of plants tested and numerator, number infected. Several figures in the same space show the results of several similar experiments. No controls became infected.
[b] Source plants graft inoculated.
[c] Source plants insect inoculated.
[d] Source plants naturally infected.
[e] Source plants mechanically inoculated.

of *Ipomoea purpurea*, 10 of the Ipomoea test species, and 2 of *I. alba* L. were inoculated from each virus source, either mechanically or by grafting. Judged by the severity of the symptoms only, they fell into the same 6 groups as when compared in sweet potato. Symptoms caused by virus A in these hosts were intermediate between those due to strains 4 and 5 of virus B. A virus occurring naturally in *Ipomoea leari Paxt.* gave, in these hosts and in sweet potato, symptoms similar to those caused by strain 2 of virus B.

Plant species susceptible to virus B.—The results of a search for alternative hosts of severe strains of this virus are summarized in Table 2. The proportion of test plants that became infected was often very small, and a species might be successfully inoculated by 1 or 2 methods but not by all. When the virus was transmitted mechanically, a species was often more readily infected with inoculum from 1 source than from another.

The failure of white flies to transmit could often be accounted for by their refusal to feed on a host other than that on which they had been reared. Negative results are therefore not conclusive.

Some effects of virus B on alternative hosts.—Leaves of all the following plants were examined for virus inclusion bodies but none was found.

1) *Gomphrena globosa.*—This was the only host found in which the virus was confined to local lesions. At first, each lesion consisted of a tiny purplish-red spot; this gradually enlarged and the centre became necrotic. If close to a vein, the lesion would extend along it; otherwise it usually became oval, and the long axis (parallel to the midrib) reached about ½ in. in length. The necrotic centre was then surrounded by a dark red halo about 1/16 in. wide. That the lesions contained active virus was shown when some were cut out and juice extracted from them was rubbed on other plants of the same species and to the Ipomoea test species. Noninoculated leaves on inoculated plants showed no symptoms, and no virus was recovered from them.

2) *Ipomoea alba.*—A very faint mottle appeared on a few young leaves, but after about a month the plants ceased to produce any symptoms. They became symptomless carriers of the virus, which was recovered from some, but not all, parts of the plants.

3) *I. dasysperma.*—Plants of this species were severely affected by the virus. The plants were much stunted and had short internodes and small distorted mottled leaves. Flower buds were formed but fell without opening.

4) *I. fistulosa.*—Infected plants were not seriously affected by the virus, which caused only a slight stunting. The young leaves were faintly mottled and distorted.

5) *I. garckeana.*—Infected plants were slightly stunted and leaves were faintly mottled.

6) *I. leari.*—One plant of this species was grown from a cutting and was apparently healthy; however, when further cuttings were taken from it, some of them showed occasional yellow patches on a few leaves. Scions from the abnormal plants grafted to other species caused symptoms similar to those caused by severe strains of virus B. The virus was not fully systemic in the host but could sometimes be recovered from plants that showed no symptoms. This virus is possibly the same as virus B.

7) *I. purpurea.*—The first symptom was usually a clearing of the veins in the young leaves; this developed later into a bright green and yellow mosaic pattern. Leaves often were distorted, and infected plants were considerably stunted.

8) *Ipomoea test species (near I. purpurea).*—Symptoms were similar to those of *I. purpurea.* Of 730 seedlings grown from seed from infected plants, all were apparently healthy (Fig. 1).

9) *Mina lobata.*—About 2 weeks after mechanical inoculation, necrotic lesions developed on the inoculated leaves; on the following day, areas surrounding the larger veins of the young leaves were bright yellow. This vein-clearing rapidly developed into a very distinct mottle. About 4 weeks after inoculation, the inoculated leaves had become completely necrotic and had fallen off. A systemic mottle was then present on all the young parts of the stunted plants.

10) *Datura ferox.*—This was very severely affected by the virus. The first symptoms appeared in the young leaves where vein-clearing rapidly developed into a bright mottle. Leaves developing later were increasingly distorted, crinkled, and misshapen: some were reduced to little more than a midrib (Fig. 3). The corolla was often broken in colour and sometimes the flowers failed to complete their development. Seed was collected from infected plants, however, and 54 seedlings grown from it showed no symptoms.

11) *Lycopersicum esculentum.*—This plant was only mildly affected by the virus, which sometimes caused only a slight general chlorosis and slight stunting. In other plants, a faint mottle, usually confined to light green patches near the base of the midrib, developed (Fig. 2).

12) *Nicotiana glutinosa.*—Infection with virus B caused rosetting of this species. Leaves developing after inoculation never exceeded 1 in. in length and were lanceolate instead of cordate as in the healthy plant. Internodes were so short that, after 4 months, the infected plants were about 6 in. high whilst healthy controls were 3–4 ft. (Fig. 4). Flower buds fell without opening.

13) *N. tabacum, White Burley type.*—When symptoms first appeared, they seemed very severe and consisted of vein-clearing, crinkling, and distortion of the leaves, especially at the bases. As the leaves increased in size, the vein-clearing was replaced by a dark green vein-banding up to ½ in. in width along each side of the main veins, with light green areas between. The crinkling disappeared, but the leaves became back-rolled at the margins. After about 2 months, the plants were only slightly chlorotic and stunted compared with the healthy controls. More than 6000 seedlings, grown from seed from infected plants, were all apparently healthy.

TABLE 2.—*Results of transmission tests to determine susceptibility of plant species to infection with virus B*

Species tested[a]	Source of inoculum	Method of transmission	No. plants infected[b]	Time[c] (days)
AMARANTACEAE				
Gomphrena globosa L.	*G. globosa*	Mechanical	30/30[d]	9–10
	Ipomoea batatas	100 *Bemisia tabaci*	6/20[d]	20
	Ipomoea test sp.	Mechanical	4/18[d]	10–15
	Petunia sp.	Mechanical	6/18[d]	10–15
CHENOPODIACEAE				
Chenopodium amaranticolor Coste & Reyn.	*I. batatas*	100 *B. tabaci*	0/9	
	Ipomoea test sp.	Mechanical	0/3,0/3,0/3	
COMPOSITAE				
Bidens pilosa L.	*G. globosa*	Mechanical	0/4	
	Ipomoea test sp.	Mechanical	0/4,0/3	
	Mina lobata	Mechanical	0/4	
	Petunia sp.	Mechanical	0/4	
Vernonia sp.	*I. batatas*	100 *B. tabaci*	0/2	
	Ipomoea test sp.	Mechanical	0/3	
	Nicotiana glutinosa	Mechanical	0/3	
	Petunia sp.	Mechanical	0/3	
Zinnia sp.	*I. batatas*	100 *B. tabaci*	0/4	
	Ipomoea test sp.	Mechanical	0/3,0/3,0/3	
	N. glutinosa	Mechanical	0/3,0/3	
	Petunia sp.	Mechanical	0/3,0/3	
CONVOLVULACEAE				
Ipomoea alba L.	*I. batatas*	Graft	9/9	14–18
	I. batatas	100 *B. tabaci*	10/28	59–69
I. dasysperma Jacquin.	Ipomoea test sp.	Mechanical	3/3,3/3	14–20
	N. glutinosa	Mechanical	0/3	
	Petunia sp.	Mechanical	0/3	
I. fistulosa Mart. ex Choisy	*I. batatas*	Graft	6/6,5/5	21–24
	I. batatas	100 *B. tabaci*	1/13	65
	Ipomoea test sp.	Mechanical	0/3,0/3	
	N. tabacum	Mechanical	0/3	
	Petunia sp.	Mechanical	0/3	
I. garckeana Vatke.	*I. batatas*	Graft	6/6	26–34
I. purpurea	*I. batatas*	20 *B. tabaci*	2/2,0/4,3/5,3/8	54–67
Ipomoea test sp.	*I. batatas*	50 *B. tabaci*	3/8	70
	I. batatas	20 *B. tabaci*	4/8,4/11,2/4	52–73
Merremia tridentata (L.) Hallier f. subsp. *alatipes* (Dammer) Verdcourt in ed.	Ipomoea test sp.	Mechanical	0/3,0/3	
Mina lobata Cerv.	Ipomoea test sp.	Mechanical	3/3,2/3,1/3	12–16
EUPHORBIACEAE				
Manihot esculenta Crantz. var. Mbarika	*I. batatas*	60 *B. tabaci*	0/4	
	I. batatas	100 *B. tabaci*	0/4,0/5,0/3	
MALVACEAE				
Abutilon sp.	Ipomoea test sp.	Mechanical	0/1,0/3	
	N. tabacum	Mechanical	0/3	
	Petunia sp.	Mechanical	0/1	
Abutilon hybrids	Ipomoea test sp.	Mechanical	0/1	
	N. tabacum	Mechanical	0/1	
	Petunia sp.	Mechanical	0/1	

TABLE 2.—*Continued*

Species tested[a]	Source of inoculum	Method of transmission	No. plants infected[b]	Time[c] (days)
Althaea rosea Cav.	*I. batatas*	100 *B. tabaci*	0/9	
	Ipomoea test sp.	Mechanical	0/3,0/3	
	M. lobata	Mechanical	0/3	
	N. tabacum	Mechanical	1?/3	
	Petunia sp.	Mechanical	0/3	
Gossypium sp. var. Bar 4/16	*I. batatas*	100 *B. tabaci*	0/8	
	I. leari Paxt.	Mechanical	0/3	
	Ipomoea test sp.	Mechanical	0/3,0/3	
	N. glutinosa	Mechanical	0/3	
	N. tabacum	Mechanical	0/3	
	Petunia sp.	Mechanical	0/3,0/3	
Hibiscus esculentus L.	*I. purpurea*	Mechanical	1?/3	
	Petunia sp.	Mechanical	0/3	
SOLANACEAE				
Datura ferox L.	*D. ferox*	Mechanical	3/3	14
	I. batatas	100 *B. tabaci*	2/10	65–68
	Ipomoea test sp	Mechanical	0/3,1/3,1/3,1/3	28–34
Lycopersicum esculentum Mill.	*G. globosa*	Mechanical	0/4	
	I. batatas	100 *B. tabaci*	1/10	72
	Ipomoea test sp.	Mechanical	0/4,1/3,1/3	30–32
	L. esculentum	Mechanical	3/3,2/3	18–23
	M. lobata	Mechanical	1/3	28
	N. glutinosa	Mechanical	2/3	24–25
	N. tabacum	Mechanical	3/3	22–25
	Petunia sp.	Mechanical	1/4,0/3	23
	Petunia sp.	Graft	10/10	21–28
Nicotiana glutinosa L.	*I. batatas*	100 *B. tabaci*	0/5	
	Ipomoea test sp.	Mechanical	0/3,2/3,1/3	11–13
	N. tabacum	Mechanical	3/3,2/3	10–15
	Petunia sp.	Mechanical	3/3	12–14
N. tabacum L. White Burley type	*I. batatas*	100 *B. tabaci*	0/6	
	Ipomoea test sp.	Mechanical	0/3,0/3,1/3,1/3	15–21
	Petunia sp.	Mechanical	3/3	15–17
Petunia sp.	*I. batatas*	Graft	5/5	30–40
	I. batatas	Mechanical	1/3,1/3,0/3,2/3,0/3,2/3	39–50
	I. batatas	100 *B. tabaci*	2/25	90
Physalis peruviana L.	Ipomoea test sp.	Mechanical	2/3	28–30
	N. glutinosa	Mechanical	2/3	28–30
	Petunia sp.	Mechanical	0/3	
	Petunia sp.	Graft	10/10	21–30

[a] I am grateful to Dr. B. Verdcourt who identified all members of Convolvulaceae used and also checked the nomenclature used in this column.

[b] Denominator shows number of plants tested and numerator the number infected unless otherwise indicated. Several figures on the same line show results of several experiments. When transmission was by grafting, apparently successful unions only are recorded. No test plant was recorded as infected until virus had been recovered from it: 2 abnormal plants from which virus was not recovered are marked "?". No controls became infected.

[c] Time elapsing between inoculation and appearance of symptoms.

[d] Denominator shows number of leaves rubbed and numerator those that produced local lesions.

14) *Petunia sp.*—The test plants were from a clone with dark magenta-coloured flowers. Both *Petunia* sp. and sweet potato are easy subjects for grafting, and apparently successful unions were obtained although they are unrelated. The sweet potato scions remained alive until they were put into bright sunshine about 2 months after grafting. It is possible that the union was not a true one and that the virus passed across mechanically. Infection resulted in mottling of the leaves, which were slightly smaller than normal, and in a slight stunting of the whole plant. The most striking feature of the infection was a breaking of the colour of the flowers, which showed many white streaks and patches (Fig. 5).

15) *Physalis peruviana.*—This species was tested because plants, apparently virus-infected, had been seen in the field growing in close proximity to severely infected sweet potatoes. It is not known if the naturally occurring virus is the same as virus B to which the species is susceptible. Symptoms of virus B were relatively mild, consisting of a slight mottling and crinkling that usually were obvious only in the young leaves. Stunting was usually slight.

16) *Other species.*—One plant only of each of 2 further species developed symptoms after inoculation. As no virus was recovered from them, the abnormalities were thought not to be due to infection. One of these plants was *Althea rosea*, which produced faintly

mottled and distorted bracts. The other was a very stunted plant of *Hibiscus esculentus;* it produced only 2 fruits, 1 of which was distorted.

Failure to transmit cassava mosaic virus to sweet potato.—Sweet potatoes infected with virus B and mosaic-affected cassava *(Manihot esculenta)* were often found growing intermingled in native plantations. As both viruses are transmitted by white flies, it seemed possible that they were identical. White flies were reared on the diseased cassava and then transferred to the sweet potato variety Muguga C. Ten white flies on each of 16 plants, 20 on each of 24 plants, and 100 on each of 10 plants failed to transmit a virus to sweet potato. Attempts to transmit virus B to cassava also failed (Table 2).

Transmission of virus A.—In addition to the species of *Ipomoea* recorded above, this virus also infected *I. garckeana*, causing very slight stunting and mottling. In addition to the plants reported in Table 1 as not becoming infected when mechanically inoculated, 6 plants of *Gomphrena globosa* also remained healthy when mechanically inoculated with juice from sweet potato as did 6 inoculated with juice from the Ipomoea test species. Attempts to transmit the virus to 10 plants of *Nicotiana tabacum* and to 10 of *Lycopersicum esculentum* by means of 10 viruliferous aphids on each plant all failed.

DISCUSSION.—The possible relationships of these 2 viruses to those affecting sweet potatoes in other countries have already been discussed (5). It then seemed possible that virus A was identical with cucumber mosaic virus. This now seems unlikely because the insect vector failed to transmit virus A to tobacco or to tomato, both of which are hosts of cucumber mosaic virus.

Virus B is distinguished by its vector from all other sweet potato viruses that had been studied in detail. Viruses known to be transmitted by white flies are few. Two such viruses attack members of the Euphorbiaceae, causing cassava mosaic disease (7) and mosaic of *Euphorbia prunifolia* Jacq. (2). All attempts to transmit virus B to cassava or to transmit cassava mosaic virus to sweet potato failed. *Lycopersicum esculentum* and all species of *Ipomoea* and *Physalis* tested are susceptible to virus B, but Costa and Bennett (2) failed to transmit the virus causing mosaic in Euphorbia to *L. esculentum* or to unnamed species of *Ipomoea* and *Physalis*. Although many malvaceous species are attacked by white-fly-transmitted viruses, there are probably only 3 viruses concerned; these cause cotton leaf curl (3), Abutilon mosaic (4), and yellow vein mosaic of *Hibiscus esculentus* (1). Attempts to transmit virus B to hosts of these 3 viruses failed. The virus causing tobacco leaf curl (6) is transmitted by white flies. Tobacco is susceptible to virus B also, but the symptoms caused are quite unlike those of leaf curl. Moreover, *Vernonia* sp., known to be a host of tobacco leaf curl virus, was unaffected by Virus B. Recently, white-fly-transmitted viruses attacking legumes (9) and cucurbits (8) have been reported from India. Neither of these viruses is sap-transmissible. It must therefore be concluded that virus B of sweet potato is distinct from any white-fly-transmitted virus previously described.

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CHEMICAL STIMULATION OF GERMINATION OF SPORES OF PIRICULARIA ORYZAE [1]

Robert L. Weintraub, William E. Miller, and Edward J. Schantz

SUMMARY

Germination on agar of spores of *Piricularia oryzae* Cav., the causal organism of rice blast, was accelerated by a factor present in rice polish and in rice oil. Similar activity was shown also by peanut and okra seed oils. The active factor was found in the unsaponifiable fraction of the rice oil. A number of pure compounds including sterols, steroidal sapogenins, steroidal amines, and α-tocopherol possess stimulatory activity. The stimulator accelerated the formation of germ tubes but did not increase the fraction of the population that ultimately germinated. A germination accelerator was demonstrated in the dew and in the guttation liquid collected from rice leaves, suggesting that such materials may play a natural ecological role. The rice-oil factor and some sapogenins accelerate germination of urediospores of the wheat stem rust fungus also.

During studies on the influence of environmental factors on germination of conidia of *Piricularia oryzae* Cav., the causal organism of rice blast, it was observed that germination was more rapid on a rice-polish agar substrate than on water agar. A subsequent study of certain aspects of the phenomenon is described in this report.

MATERIALS AND METHODS.—All experiments were carried out with a single batch of spores; this was divided initially into a large number of sublots, each of which was sealed in a glass ampoule under nitrogen and stored at 4°C until needed. A fresh ampoule was used for each experiment. With this technique, a high degree of reproducibility was achieved and germinability remained substantially unaltered for more than a year.

Ten ml of 1 per cent Difco agar in Syracuse watch glasses was used as substrate for germination. A uniform deposit of spores was obtained by means of a settling tower.[2] As determined in preliminary trials, a sufficiently low concentration of spores was employed to obviate any inhibitory effect due to high spore density. The dishes were incubated in darkness in a water-saturated atmosphere at 25°±0.1°C. At least 1 liter of incubator volume was provided per dish. After the desired interval, the dishes were removed and sprayed with 37 per cent formaldehyde solution to stop further development and to permit subsequent examination and counting when convenient. Spores with 1 or more germ tubes of length as great as the spore width were counted as germinated. In general, 5 areas, each of 100 spores, were counted on each of 2 dishes.

EXPERIMENTAL RESULTS.—As shown by the typical progress curve in Figure 1, germination on water agar commenced about 1 hour after seeding and virtually all germinable spores had germinated within 8 hours. Results on agar prepared with tap water were indistinguishable from those on agar prepared with distilled water.

In contrast, germination was accelerated markedly on rice-polish agar; the percentage of germinated spores at 2 hours was approximately twice that on water agar. The rice-polish agar was prepared by autoclaving a mixture containing 2 g commercial rice polish and 1 g agar in 100 ml distilled water and straining the hot suspension through cheesecloth.

The stimulating factor in the rice polish was extractable by hot water. Two g of rice polish was steeped 1 hour in hot water after which the solids

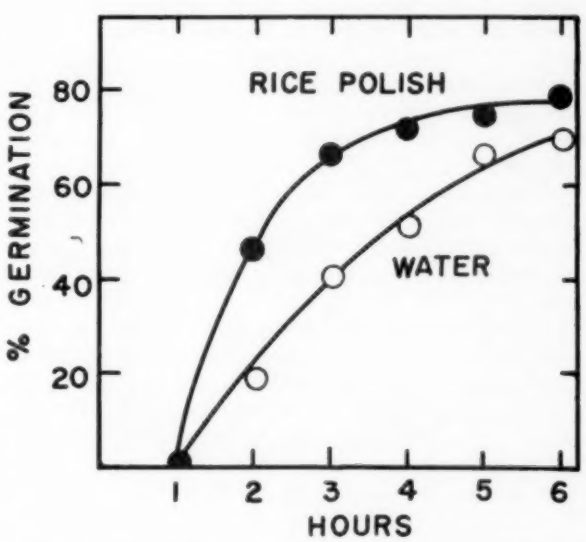

FIG. 1. Time course of germination of spores of *Piricularia oryzae* on water agar and on rice-polish agar.

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The several seed oils were obtained through the kindness of H. P. Newton and C. H. Fisher of the U. S. Department of Agriculture. The steroids were supplied through the generosity of M. E. Wall, U. S. Department of Agriculture, and H. P. Doukas of Fort Detrick.

[2] Bell, F. H., C. G. Schmidt, W. E. Miller, and C. H. Kingsolver. 1952. A technique for obtaining uniform deposition of urediospores on cereal leaves. Phytopathology 42: 340.

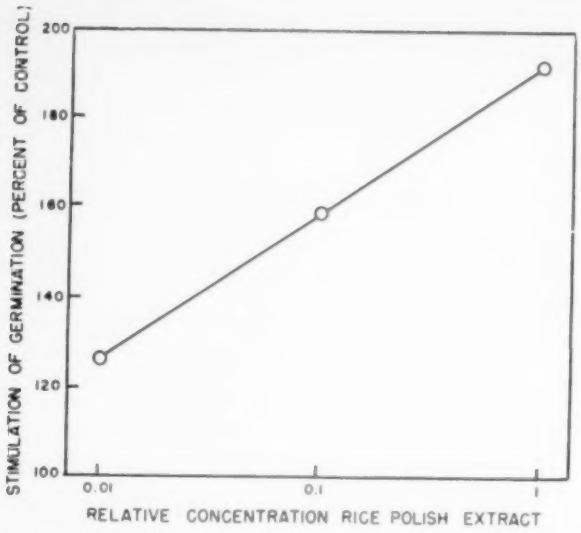

FIG. 2. Acceleration of germination of spores of *Piricularia oryzae* by rice oil extract as a function of its concentration. The highest concentration shown is equivalent to that in a saturated aqueous solution.

were removed by decantation and centrifugation. The clear supernatant was active. The effectiveness of the extract varied exponentially with its concentration (Fig. 2).

An oily film was invariably present on the surface of the aqueous extracts from rice polish. To ascertain whether the stimulatory factor was present in the oil or the water, the 2 phases were separated and tested individually. Both were found to be active, suggesting that the factor was present initially in the oily fraction of the rice polish from which it was partially extracted by the water.

This observation suggested that the stimulator might be present in commercial rice oil. Tests were made with samples of both crude and refined rice oil. After the oil was shaken in water to form a saturated aqueous solution, excess oil was removed by filtration through filter paper and the filtrate mixed with agar. Both the crude and refined samples contained the active factor.

Similar tests were then carried out with a number of other seed oils. Peanut and okra oils also were found to contain a stimulatory factor, whereas the results with cottonseed and sesame oils were inconclusive.

The stimulator accelerated the formation of germ tubes (Fig. 1); it did not increase the fraction of the population that ultimately germinated. A limited number of measurements of growth rates of individual germ tubes indicated that the acceleration of germ tube initiation was not followed by an increased rate of the subsequent elongation. Neither did the rice-oil factor influence significantly the number or distribution of germ tubes produced by the tricellular Piricularia spore.

Quantitatively, the active factor is a very minor constituent of the whole-seed oil. It is soluble in water to some extent, but its partition in a water-oil system is in favor of the oil. It is relatively thermostable, as it can be autoclaved without complete inactivation. Some aqueous solutions retained their activity during several weeks of storage at room temperature.

As determined by the capillary-rise method, the surface tension of a saturated aqueous extract of rice oil was identical with that of pure water; however, extracts of rice oil do contain a surface-active material, as indicated by their much greater ability to wet rice leaves than has pure water. In contrast, spores of Piricularia appeared to be wetted as readily by pure water as by the rice-oil extract. Furthermore, no stimulation of germination was induced by a synthetic surfactant, Tween 20 (a polyoxy-alkaline derivative of sorbitan monolaurate manufactured by Atlas Powder Co.), at concentrations of 0.2, 0.02, or 0.002 per cent in agar. Hence, there seems to be no relation between the germination stimulator and the surface-active constituent of the rice oil.

To ascertain whether the activity was associated with the saponifiable or unsaponifiable constituents of rice oil, 50 g of oil was mixed with 500 ml of 0.5 N KOH in 95 per cent ethanol and refluxed for 3 hours. After cooling, the saponified oil was diluted with 1 to 2 volumes of water and extracted with peroxide-free ethyl ether to separate the ether-soluble substances from the potassium salts of the fatty acids. The use of peroxide-free ether was indicated because the activity was lost when the product was dissolved and allowed to stand in old ether but not in freshly distilled ether.

Two procedures were employed in further fractionating the unsaponifiable constituents, which comprise 3–6 per cent of the oil.[3]

In the first method, the pigmented ethyl ether solution was washed with water and the ether allowed to evaporate. The residue was taken up immediately in just enough petroleum ether or *n*-hexane to dissolve it. The solution was cooled to about −70° C in a mixture of solid CO_2 and acetone, and the insoluble white waxy material that precipitated was collected. When the volume of the petroleum ether was reduced to about 1 ml and cooled, practically all of the waxy material could be removed whereas the pigments remained in solution. The insoluble material was redissolved and again cooled to remove any remaining traces of pigment. The solvent was then evaporated, and the residue (about 700 mg) was stored under nitrogen.

Bioassay of the several fractions showed that the activity was present solely in the waxy material, whereas the pigments and the saponifiable portion of the oil were entirely inactive.

[3] McCall, E. R., C. L. Hoffpauir, and D. B. Skau. 1951. The chemical composition of rice. A literature review. U. S. Dept. Agr. Publ. AIC-312, 49 p.

A second method of fractionation of the ether-soluble, unsaponifiable substances utilized paper chromatography. About 20 mg of the material in ethyl ether solution was streaked on a 2 × 18-in. strip of Whatman No. 1 filter paper. After the paper had dried for a few seconds, the chromatogram was developed by descending chromatography in a commercial cabinet. Among several solvents tried for developing the chromatogram, petroleum ether was most satisfactory. After development, the paper was cut into 10 sections, each corresponding to 0.1 R_f interval; the sections were extracted separately with petroleum ether. The pigments moved at the solvent front (R_f 0.95–1.0) whereas the biologically active materials were confined to the region R_f 0.6–0.8.

The appearance and solubilities in various solvents of the white, waxy, active material suggested that it might be a sterol or steroid. Sterols are reported to constitute about 25 per cent of the unsaponifiable matter of rice oil.[3] Accordingly, several pure steroidal substances, as well as some other compounds, were tested for germination-accelerating activity. Because most of the compounds tested were of very low solubility in water, it was inexpedient, in these screening experiments, to prepare solutions of known concentration. All assays were carried out by preparing saturated solutions and diluting them with agar so that the final concentration of each compound was equal to one-tenth its saturation value. As the various compounds doubtless differed in solubility, the results (Table 1) do not provide information on their relative activities on an equal-weight or equimolar basis; they should be interpreted as showing only which substances were active or inactive at the unknown concentrations tested. Among the sterols, cholesterol was active whereas stigmasterol (a constituent of rice oil) and broccoli-leaf sterol were not. Phytosterol and dihydrositosterol, which also are constituents of rice oil,[3] were not tested. The triterpenoid sterol lanosterol was inactive. Highest activity was found in a group of steroidal sapogenins—diosgenin, gitogenin, hecogenin, isodiosgenin, sarsasapogenin, smilagenin, and ticogenin. Other sapogenins (dihydroticogenin, kryptogenin, and pseudodiosgenin) were less effective. Tomatidine, a steroidal amine, showed significant activity whereas dihydrotomatidine and solanidine did not. The only nonsteroid found to be active was α-tocopherol (vitamin E); this substance has been reported as present in rice.[3]

One or 2 points should be noted in connection with these results. A considerable variety of materials possess germination-accelerating activity. The magnitude of the maximal stimulation by these substances of known structure is the same as that induced by the rice-oil extract. It seems rather unlikely that some of these, such as the steroidal sapogenins and amines, are present in rice oil. Stigmasterol, a known rice-oil constituent, was inactive. There is some evidence, furthermore, that the stimulator from rice oil, at least in the impure condition thus far available and when in the dry state, is inactivated by oxidation by atmospheric oxygen whereas the pure sapogenins are quite stable. The nature of the rice-oil factor must be regarded as still uncertain.

TABLE 1.—*Acceleration of germination of spores of Piricularia oryzae by various substances*

Substance	Relative activity[a]
Rice-oil extract	148
Cholesterol	117
Stigmasterol	97
Broccoli-leaf sterol[b]	99
Lanosterol	101
Diosgenin	140
Hecogenin	155
Gitogenin	149
Sarsasapogenin	126
Sarsasapogenin acetate	133
Smilagenin acetate	146
Tigogenin acetate	136
Dihydrotigogenin	115
Pseudodiosgenin	118
Isodiosgenin	126
Kryptogenin	114
Tomatidine	114
Dihydrotomatidine	100
Solanidine	108
α-Tocopherol	137

[a] Activity expressed as percentage germination in 2 hours relative to water-agar control. Each value is the mean of 2 or more independent experiments.
[b] Structure unknown but probably related to β-sitosterol.

The occurrence of a germination-accelerating substance in the host raises the question of whether it may play a natural ecological role. The possible benefit to the parasite may be readily envisioned: Inasmuch as the processes of germination and penetration into the rice leaf must be completed while moisture is present on the surface, the more rapidly the germination process is initiated the shorter will be the dew period requirement for successful infection.

To ascertain whether a germination accelerator was present on the foliage, dew was induced to form on rice plants by transferring them from the greenhouse to a special chamber.[4] The dew was removed by gently blotting the leaves with filter paper, the paper was extracted with water, and the aqueous extract mixed with agar for bioassay. The results of several experiments are assembled in Table 2. In all cases, the dew collected from plants within 5 to 16 hours after they were placed in the chamber contained a highly active factor. A second collection made after 1 or 2 additional days in the nonilluminated chamber usually was devoid of activity.

The moisture blotted from the leaves in the foregoing experiments was presumed to originate in part from condensation of atmospheric water vapor and in part from liquid excreted by guttation of the leaves. In order to establish whether the active factor is

[4] Mitchell, J. E., and E. Cherry. 1954. A variable temperature incubation chamber permitting controlled deposition of dew. Phytopathology 44: 498.

excreted in the guttation liquid, potted plants were placed in the chamber and warm water was added to the soil. Guttation droplets began to appear within ½ hour. After a few hours, they were removed either with filter paper (care was taken to avoid touching the leaf surface) or with a very fine micropipette. Three such experiments showed the guttation liquid to have high germination-accelerating activity. As in the earlier dew experiments, the guttation liquid collected from plants after 1 or 2 days in darkness was inactive.

No evidence is available on the possible identity of the factor on the rice leaf with that demonstrated in the rice oil.

To ascertain whether the stimulatory factors affect the germination of other species of fungal spores, tests were made with urediospores of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. et E. Henn. and with conidia of *Helminthosporium* sp. The germination technique described above was used. The response of the spores of the wheat stem rust fungus was as great as that of the spores of the rice blast fungus, or greater; consistent stimulation was not observed in *Helminthosporium* sp.

TABLE 2.—*Germination of spores of Piricularia oryzae in dew collected from rice leaves*[a]

Expt.	Dew period	Germination in 2 hours as percentage of control[b]
1	16 Hours	139
	Same plants 2½ days later	152
2	16 Hours	142
3	5 Hours	167
	Same plants 23 hours later	106
4	6 Hours	130
	Same plants 24 hours later	110
5	6 Hours	139
	Same plants 24 hours later	109

[a] Five- to 6-week-old potted rice plants of variety C.I. 8970 were used.

[b] The control agar was prepared with an extract of filter paper that had not been in contact with rice plants.

TABLE 3.—*Acceleration of germination of spores of Puccinia graminis*

Substance	Spore lot	Expt.	Germination in 2 hours as percentage of water-agar control
Rice-polish extract	W-18	1	162
Rice-oil extract	W-31	1	200
		2	165
Diosgenin	W-56-6-11	1	141
		2	200
		3	260
	W-31	1	136
		2	126

DISCUSSION.—The literature contains numerous reports of the improvement of germination of various fungal spores by a miscellany of natural products and extractives; several citations are given in the review of Gottlieb.[5] In many of these reports, the description of techniques is insufficient to make clear whether the effect is a true increase in amount of germination or merely a transient acceleration as in the present instance. Although a number of known compounds of diverse nature have been found to benefit germination, the steroids do not appear to have been implicated previously.

Virtually nothing is known concerning the mechanism of action of the accelerator of Piricularia germination. It does not counteract the retardation of germination that is brought about by exposure to light of moderately high intensity.

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[5] Gottlieb, D. 1950. The physiology of spore germination in fungi. Botan. Rev. 16: 229-257.

THE EFFECTIVENESS OF CERTAIN ERADICANT FUNGICIDES ON INHIBITION OF GEMMAE OF MYCENA CITRICOLOR [1]

Eddie Echandi and R. H. Segall [2]

SUMMARY

"Ojo de gallo" disease of coffee was controlled by the use of certain mercury-containing fungicides. Three dry-season applications of mercury fungicides controlled the disease as well as, or better than, did 9 applications of copper fungicide made both in the dry and in the rainy season. Dry-season applications of mercury fungicides were found to be more effective than those made in the rainy season. Use of this program resulted in the presence of a toxic mercury residue within the harvested coffee beans, making the program unsuitable for use in the practical control of the disease.

"Ojo de gallo" or American leaf spot caused by *Mycena citricolor* (Berk. & Curt.) Sacc. (=*Omphalia flavida* Maubl. & Rang.), a member of the family Agaricaceae of the Basidiomycetes, is the most important disease of coffee trees in the Western Hemisphere. Estimates indicate that 20 per cent of the Costa Rican coffee crop is lost annually as a result of this disease (5). Losses are due principally to excessive drop of affected leaves and berries.

Inoculum is distributed by means of gemmae borne on pedicels that develop on the surface of small circular necrotic spots (0.5–1 cm in diameter) on leaves (Fig. 1, A), fruits, stems, and young branches. Relatively small numbers of gemmae, usually 1–20 at any 1 period and less than 100 per season, are formed on each spot (Fig. 1, B). They are spread from tree to tree by splashing rain drops; they are too heavy to be borne by air currents (6). Long distance dissemination is principally by gemmae that stick to insects, birds, workers, etc.

Control methods such as heavy pruning, defoliation of diseased trees, reduction of shade, improvement of soil drainage, and fertilization have been recommended, but none of these have given satisfactory control. Bordeaux mixture was one of the first fungicides used for controlling the disease, but early workers claimed that satisfactory results were not obtained and that applications were too expensive and difficult. Recently, Pérez (3) tried other copper fungicides and obtained promising results. For this reason, copper fungicides are now widely used in Costa Rica. Use of these and other fungicides for the control of coffee diseases has been reviewed recently by Wellman (7).

Since rainfall in the Costa Rican coffee zone is approximately 100 in. per year, it is difficult to maintain an adequate film of fungicide on the foliage. Spray applications more frequent than once a month are not considered economically feasible by most Costa Rican coffee growers. Control of "ojo de gallo" in certain areas by the use of protectant fungicides has, therefore, not been feasible. Investigations by Echandi (2) in 1956 showed that certain eradicant fungicides would inhibit formation of gemmae on coffee leaf lesions. Because of the relatively small number of gemmae, their large size, and slow spread, it was thought that the disease might be eliminated from coffee farms by the application of a fungicide that would inhibit sporulation. This paper presents experimental evidence that demonstrates the feasibility of this mode of attack; however, where mercury containing fungicides were used, a residue was found in the beans. Therefore these fungicides should not be used for the control of "ojo de gallo" disease in coffee plantations, as a mercury residue will probably result.

MATERIALS AND METHODS.—Fungicides found by Echandi (2) to be effective in inhibiting gemmae formation were selected for field applications. Heavily diseased trees of *Coffea arabica* L. growing on the Central Plateau, the main coffee-growing area of Costa Rica, were chosen for fungicidal tests. The number of trees per treatment varied from 1 to 16 in various experiments, and all treatments were replicated 3 times. Special care was taken to spray foliage until the fungicide solution started to run off. Fungicides were applied at monthly intervals. Species of *Inga* commonly used to shade coffee trees are hosts of *Mycena citricolor*. Since these shade trees are too large to be effectively sprayed, they were heavily pruned before start of the fungicide applications to remove possible diseased leaves. The following fungicides were used in field trials: 1) Emmi, 90% 1, 4, 5, 6, 7, 7-hexachloro-*N*-(ethylmercuri)bicyclo[2.2.1]hept-5-ene-2,3-dicarboximide; 2) Puratized Agricultural Spray, 7.5% tris(2-hydroxyethyl) (phenylmercuri)ammonium lactate; 3) Phix, 22% phenylmercury acetate; 4) Hostaquick, 5% phenylmercury acetate; 5) Tri-Basic, 98% basic copper sulfate; 6) Cupravit, 85% basic copper chloride; and 7) Elgetol, 19% sodium 4,6-dinitro-*o*-cresoxide. Other fungicides containing mercury or arsenic were tested in the laboratory and in small field plots. In all cases, the

[1] Accepted for publication July 23, 1957.

[2] Respectively, Professor of Plant Pathology, University of Costa Rica, and Plant Pathologist, United States Operations Mission to Costa Rica, International Cooperation Administration.

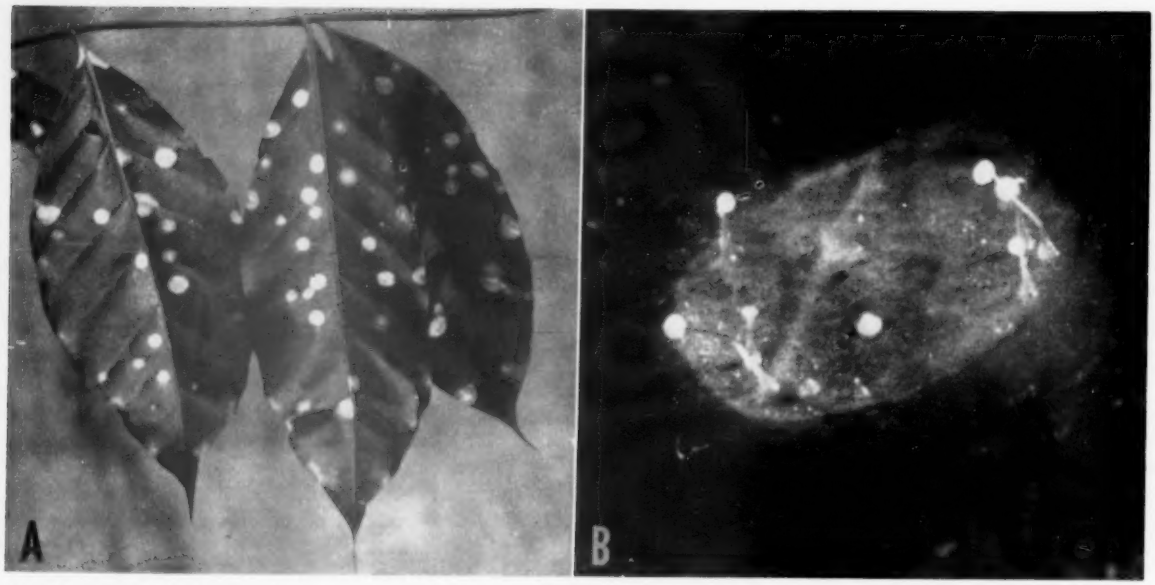

FIG. 1. A) Coffee leaves showing typical leaf spots of the "ojo de gallo" disease. B) Gemmae of *Mycena citricolor* formed on a leaf spot caused by the fungus.

spreader stickers. Film Fast (a mixture of organic gums and calcium caseinate) or Triton B1956 (77% phthalic glyceral alkyl resin in ethylene dichloride) were added to the fungicidal mixtures. Data were obtained by counting the total number of spots, the number of spots containing gemmae, and the number of gemmae per spot on 40 randomly selected diseased leaves from both treated and untreated plots. In addition, plants in each plot were rated at the end of the rainy season for disease intensity according to the method of Sequeira (4). Data from all experiments were transformed to angles (1) and analyzed statistically.

During the dry season, which normally extends from January to April, humidity in coffee plantations is relatively low; spread of the disease is slow since few or no gemmae are formed. In experiments started in March, 3 applications of eradicant fungicides were made at monthly intervals. For comparison, protectant fungicides were applied on comparable trees; these were started at the same time and continued throughout the rainy season for a total of 9 sprays. These fungicides were applied through power sprayers at a pressure of approximately 150 lb. per sq. in. and at a rate of 250 gal. per acre.

In experiments initiated during the rainy season in September, applications were made with knapsack sprayers or with low-gallonage power applicators.

TABLE 1.—*Gemma formation and disease intensity on coffee plants sprayed with fungicides during the dry season for control of "ojo de gallo"*

Fungicide[a]	Concentration per 100 gal.	Average percentage[b] of spots with gemmae			Disease intensity[b, c] at end of rainy season
		June	October	November	
Puratized Agr. Spray	0.84 pint	0.0**	1.3**	22.9**	4.5**
Emmi	0.88 lb.	0.0**	0.0**	0.0**	0.0**
Phix	0.88 lb.	0.0**	1.5**	25.9**	6.4**
Hostaquick	0.88 lb.	0.0**	7.6**	31.6**	20.0**
Elgetol	0.88 lb.	36.8**	63.5	76.6	43.9
Tri-Basic	4.00 lb.	66.7	57.4	63.6	15.9**
Cupravit	2.00 lb.	67.3	58.0	68.1	27.2**
None		72.3	64.5	78.7	55.6

[a] Applications of eradicant fungicides made in March, April, and May. Applications of protectant fungicides made at monthly intervals extending from March through November.

[b] Single and double asterisks indicate that differences between indicated percentages and percentages of unsprayed checks are significant at 0.05 and 0.01 levels, respectively.

[c] Disease rating $= \frac{\text{Sum of numerical ratings} \times 100}{\text{Total number of plants in plot} \times 5}$ Numeral 5 in formula is rating of maximal disease category.

TABLE 2.—*Gemma formation and disease intensity on coffee plants sprayed with 3 concentrations of mercury fungicides during rainy season with high-gallonage equipment for the control of "ojo de gallo"*

Fungicide[a]	Concentration per 100 gal.	Average percentage[b] of gemmae 1 month after indicated application		Disease intensity[b,c] at end of rainy season
		First	Second	
Puratized Agr. Spray	1.26 pint	18.4**	13.1**	13.3**
	0.84 pint	40.5**	10.3**	20.0**
	0.63 pint	65.7**	24.3**	40.0**
Emmi	0.88 lb.	0.0**	0.0**	0.0**
	0.66 lb.	2.1**	0.0**	0.0**
	0.44 lb.	13.5**	0.8**	0.0**
Hostaquick	1.32 lb.	33.6**	20.1**	40.0**
	0.88 lb.	63.7	38.6**	66.7**
	0.66 lb.	72.9	53.1	73.3*
None		84.1	79.5	100.0

[a] Applications of fungicides made in September and October.

[b] Single and double asterisks indicate that differences between indicated percentages and percentages of unsprayed checks are significant at 0.05 and 0.01 levels, respectively.

[c] Disease rating $= \frac{\text{Sum of numerical ratings} \times 100}{\text{Total number of plants in plot} \times 5}$. Numeral 5 in formula is rating of maximal disease category.

Three eradicant fungicides, each at 3 different concentrations, were applied twice at monthly intervals. Data on gemma formation were taken a month after each spray and before any subsequent spraying.

RESULTS.—*Dry-season applications.*—Examinations of leaves in June, following the start of the rainy season and a month after the third spray, indicated that no gemmae were produced on leaves of plants sprayed with any of the mercury-containing fungicides (Table 1). Plants sprayed with any of the other fungicides had leaf spots that carried large numbers of gemmae, indicating that these fungicides had little apparent inhibitory effect on gemma formation. Data taken during October showed that gemmae were produced on a few leaf spots on some plants sprayed with mercury. In November, at the close of the rainy season, mercury fungicides continued to give significantly greater inhibition of gemmae than did fungicides of other active ingredients; however, differences in the plots treated with different mercury fungicides became evident. Although Emmi was the only fungicide that under these conditions completely inhibited gemma formation, Puratized Agricultural Spray, Phix, and Hostaquick also reduced the number of gemmae by a statistically significant amount. Disease intensity ratings indicated that sprays of Emmi or of Puratized Agricultural Spray resulted in control that was significantly better statistically than that given by sprays of Hostaquick or of copper-containing fungicides. Disease intensity ratings also indicated that plants treated with copper fungicides had significantly less "ojo de gallo" than did those sprayed with Elgetol or those untreated.

Better control was obtained by 3 applications of Emmi or of Puratized Agricultural Spray than with 9 applications of a copper fungicide. In an area of the Central Plateau, where the temperature is high and both rainfall and humidity are low, 1 application of certain of the mercury fungicides during the dry season completely inhibited gemma formation and therefore eliminated the disease from the sprayed plots.

Plants throughout the plots had symptoms typical of zinc deficiency before they were sprayed. These symptoms were accentuated by treating the plants with mercury fungicides. To determine if the deficiency symptoms could be corrected by the application of zinc, half of the plants in each plot were sprayed with a mixture of zinc oxide (30%) and zinc sulfate (70%) at a rate of 4 lb. per 100 gal. On plants given this treatment, the severity of the deficiency symptoms on affected plants was appreciably less than on nontreated ones, regardless of the fungicide application.

Rainy-season applications.—The 3 mercury fungicides used, Emmi, Puratized Agricultural Spray, and Hostaquick, controlled "ojo de gallo" (Tables 2, 3). Defoliation that takes place during the rainy season was either eliminated or slight on all sprayed plots. On plants sprayed with high-gallonage equipment (Table 2), gemma formation was inhibited completely after the first spray with Emmi at the highest concentration. Following the second spray, Emmi at all concentrations completely inhibited gemmae, and the number of spots with gemmae decreased with all 3 fungicides. Statistically significant differences in both gemma formation and disease intensity were noted among the 3 mercury compounds used and, in some cases, among different levels of concentration.

Results with low-gallonage spray trials (Table 3) were similar to those of high gallonage in that there were statistically significant differences in gemma inhibition and in disease intensity among all 3 fungicides. In another experiment, where Puratized Agri-

TABLE 3.—*Gemma formation and disease intensity on coffee plants sprayed with 3 concentrations of mercury fungicides during rainy season with low-gallonage equipment for the control of "ojo de gallo"*

Fungicide[a]	Concentration per 100 gal.	Average percentage[b] of gemmae 1 month after indicated application		Disease intensity[b,c] at end of rainy season
		First	Second	
Puratized Agr. Spray	3.2 pint	14.8**	3.3**	13.3**
	2.1 pint	19.3**	11.3**	26.6**
	1.0 pint	64.8**	9.5**	46.6**
Emmi	3.0 lb.	0.0**	0.0**	0.0**
	2.2 lb.	1.3**	0.0**	0.0**
	1.3 lb.	19.5**	0.6**	0.0**
Hostaquick	4.4 lb.	44.1**	10.9**	46.6**
	3.3 lb.	74.3*	8.3**	33.3**
	2.2 lb.	88.9	38.6**	53.3**
None		90.0	92.0	93.3

[a] Application of fungicides made in September and October.

[b] Single and double asterisks indicate that differences between indicated percentages and percentages of unsprayed checks are significant at 0.05 and 0.01 levels, respectively.

[c] Disease rating $= \frac{\text{Sum of numerical ratings} \times 100}{\text{Total number of plants in plot} \times 5}$. Numeral 5 in formula is rating of maximal disease category.

cultural Spray was applied with low-gallonage equipment at 4 pints per 100 gal., marginal burning of the coffee leaves occurred.

Zinc deficiency symptoms were also accentuated in these experiments as they were in dry-season experiments.

Other fungicides containing mercury or arsenic as active ingredients also showed promise in laboratory and small field trials.

Samples of coffee beans taken from trees sprayed in the dry and rainy seasons were analyzed in the laboratories of the United States Food and Drug Administration. Results of these tests[3] showed that, "small but significant amounts of mercury were found in the beans." Residues were found when applications were made before or during fruit formation. When trees with fruit approaching maturity were sprayed, no mercury residue was found in the bean; however, fungicidal applications when the fruit is approaching maturity, September to November, at the height of the rainy season, were found to be least effective in controlling "ojo de gallo."

DISCUSSION.—Spots of "ojo de gallo" sprayed with fungicides containing mercury became light in color and dry after a few weeks, suggesting that the fungus in the spots was dead; however, lesions on leaves close to the ground or covered with dense foliage occasionally produced gemmae. It was from these lesions that most of the new infections began during the rainy season on some sprayed trees.

The principle of the proposed method of controlling "ojo de gallo" is the inhibition of spore formation of the pathogen. The successful demonstration of the effectiveness of a method based on this principle is of particular importance in tropical areas of high rainfall, where the maintenance of a protective layer of fungicides on foliage is difficult or impossible.

Unfortunately the mercury eradicants that gave better control than did copper protectants cannot at present be used in coffee plantations because of a mercury residue within the coffee beans, making them unsuitable for commercial use. Other fungicides may be found, however, that will have similar eradicant properties without leaving toxic residues.

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AGRICULTURE AND NATURAL RESOURCES BRANCH
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[3] Office Memorandum, United States Government, Dated March 27, 1957. From: John L. Harvey, Deputy Commissioner Food and Drug Administration. To: Robert W. Tyson S/Food, ICA. Subject: Mercury Content of Coffee Beans.

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A METHOD FOR ISOLATING VIRUS-LIKE PARTICLES FROM WOODY PLANTS SHOWING SYMPTOMS OF ELM PHLOEM NECROSIS, PEACH X-DISEASE, AND CHERRY RING SPOT [1]

H. H. Thornberry

SUMMARY

A method was developed for isolating virus-like particles from foliage of woody plants with viral infections. It combined the principles of dilutive extraction, clarification of extract by filtration, concentration and partial purification by adsorption and elution, and differential sedimentation by ultracentrifugation. The dilutive extraction at pH 8.5 favored solubilization of substances, and it also lowered the viscosity of the extractable materials from the tissues. The filtration at pH 8.5 for clarification allowed solutes and negatively charged particles below the size of bacteria to pass into the filtrate, but solids the size of bacteria or larger, positively charged particles, and gummy substances were retained by the Celite cake. The Celite cake for the adsorptive filtration of the clarified filtrate adjusted to pH 3.5 retained positively charged particles and aggregates that were separated from the Celite particles by elution at pH 8.5 and by filtration through filter paper. Final preparations following differential sedimentation of such filtrates from both elm phloem necrosis and peach X-disease material contained virus-like particles 50 mμ in diameter. Similar preparations from cherry ring spot material contained 80-mμ particles. A preparation from 1 species of leafhopper (*Colladonus geminatus* Van D.) carrying the virus of western peach X-disease contained 50-mμ particles. No observable infection resulted from infectivity tests made with the preparations.

A search for procedures for the isolation of viruses or virus-like particles from diseased woody plants was undertaken for 3 reasons: 1) importance of woody-plant virus diseases, 2) necessity for purified virus in certain studies, and 3) the scanty supply of published information on this subject. It was aided by experience gained in the isolation of carnation mosaic virus (1) from diseased tissues containing a low content of virus but a high content of hydrophylic substances that interfere with the extraction and purification of this virus and in experiments on the filtration, adsorption, and elution of tobacco mosaic virus (2, 18). In the absence of suitable assays for viral infectivity, the search was guided by electron microscopy of the various fractions and preparations. This paper describes a provisional procedure based upon the findings, discusses briefly the problem and findings, and illustrates the virus-like particles isolated. An abstract of portions of the paper has been published (20). The investigations were completed prior to the appearance of 2 papers on the purification of viruses of stone fruits (11, 21).

MATERIALS AND METHODS.—The virus-containing plant and insect materials were as follows: 1) elm phloem necrosis foliage from the younger portions of mature trees of elm, *Ulmus americana* L., collected during May at Urbana, Illinois; 2) peach X-disease foliage from bushes of chokecherry, *Prunus virginiana* L., collected during September near Oak Forest, Illinois; 3) cherry ring spot foliage from bearing trees of sour cherry, *Prunus cerasus* L., collected during May near Louisiana, Missouri; 4) elm phloem necrosis virus vector, *Scaphoideus luteolus* Van D., from Ohio; and 5) peach western X-disease virus vectors, *Colladonus geminatus* Van D. and *Scaphytopius acutus* Say, from Washington. The controls were virus-free specimens of the same species from the same sources as the virus-containing materials.

Celite filter cakes of 2 sizes were prepared; one for the crude-juice clarification and the other for the adsorptive step in the isolation procedure. A slurry of approximately 10 per cent Celite powder (Johns-Manville No. 209—"Hyflow") in distilled water was filtered at approximately 635 mm Hg of negative pressure in an assembly consisting of 2 filter paper discs (Whatman No. 1) in a Buchner funnel attached to a suction flask. Approximately 100 g of air-dry Celite powder and a 15-cm Buchner funnel were used to prepare the large filter cakes for clarification, and approximately 10 g of the powder and a 9-cm Buchner funnel were used to make the small filter cakes for adsorption. The lodged Celite particles were compacted by pounding with a pestle. The handle end of the pestle was used at the junction between the cake and funnel to assure a water-tight seal. The remaining portion of the cake was compacted with the rounded end of the pestle to give maximal compactness. The compacted large and small filter cakes were approximately 12 mm and 5 mm thick, respectively.

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Phosphate buffer was used to maintain the desired pH. Unless otherwise stated, the buffer contained 0.1 M disodium hydrogen phosphate adjusted to pH 8.5 with 0.1 or 1.0 N NaOH or HCl.

Electron microscopy, with an RCA model EMB microscope at magnifications from 10,000 to 50,000, was employed to detect virus-like particles in samples of the preparation at various steps in the procedure. Mounts were shadowed with palladium.

Infectivity tests were made with some of the preparations. In the case of preparations from elm phloem necrosis, both mechanical and leafhopper-vector methods were used on succulent shoots and foliage of young elm trees. Some of the preparations from peach X-disease were applied mechanically to 143 herbaceous plants. Some of the preparations from cherry ring spot were applied mechanically to the foliage of 6 herbaceous plants, including a supposedly local-lesion host, cotyledons of cucumber.

ISOLATION PROCEDURE.—The isolation procedure consisted of 4 general steps: 1) buffer extraction of ground or triturated tissues, 2) clarification of the crude extract by filtration, 3) concentration and purification by adsorption and elution, and 4) differential sedimentation. The procedure was developed from findings from purificative experiments on the elm phloem necrosis material and was later applied to the other materials without basic modification. Trial procedures giving preparations devoid of virus-like particles were abandoned, and those revealing suspected particles were pursued and modified until samples of the preparations contained a reasonable display of particles as detected in the electron microscope.

Extraction was done on 200 g of plant tissues that had been ground while frozen or while cold in a household hand-operated food grinder with a cutting plate containing 10 orifices 1 cm in diameter. Two hundred ml of a 1.0 M solution of dibasic sodium phosphate was added to the pulp and liquid. The mixture was stirred thoroughly, adjusted to pH 8.5, and then strained through a 4-ply cheesecloth bag by wringing by hand. The strained liquid, referred to as crude extract, was diluted to a final volume of 2 liters. Triturated tissues of insects were extracted in a similar manner but on a reduced scale.

Clarification of the crude juice was achieved by filtration as soon as feasible at pH 8.5 through a large compacted Celite filter cake. This removed particles approximately the size of bacteria or larger and also most of the gummy substances. To facilitate filtration, the nonfilterable solids and gummy substances were removed from the surface of the filter cake by scraping with a spatula that had been ground to a flattened end to provide maximal scraping with minimal removal of Celite particles. When approximately 200 ml of the liquid had filtered, the filtration was disrupted for removal of the filtrate from the suction flask and for the adjustment of the liquid to pH 7.0. This was done to reduce possible denaturation of viral particles at pH 8.5 and to avoid the necessity of refiltering the filtrate if the filter cake should break. The combined filtrate was adjusted to pH 3.5 for the adsorptive filtration.

The adsorptive filtration of clarified extract was done at pH 3.5 with a small compacted Celite filter cake and suction. The filtrate was discarded, and the residue and Celite particles were resuspended in 200 ml of buffer at pH 8.5 for elution of virus and virus-like substances from the Celite particles. NaOH was added with stirring to bring the suspension to pH 8.5. The eluate was separated from the Celite particles by filtration through filter paper in a 9-cm Buchner funnel with suction. The noncompacted Celite cake was resuspended in 200 ml of new buffer at pH 8.5 and filtered as before. The filtrates were combined and adjusted to pH 7.0 for centrifugation.

The differential sedimentations consisted of conventional procedures for the purification of viruses by centrifugation.

Liquids containing an appreciable number of bacteria were filtered through Mandler bacteriological candles at pH 8.5 to remove bacteria.

The flow chart (Fig. 1) depicts the entire procedure consisting of 3 filtrations and the differential sedimentations.

RESULTS.—Particles obtained from each of the 3 diseased plant materials were distinct and virus-like in size and shape. Representative particles are illustrated in Figure 2. The particles from elm phloem necrosis material and from peach X-disease material were spheres approximately 50 mμ in diameter. Those from cherry ring spot material were spheres approximately 80 mμ in diameter. Only indistinct particles and some amorphous material were observable in mounts from healthy material. Spherical particles approximately 50 mμ in diameter were observed in preparations from a small sample of viruliferous (peach X-disease virus) leafhoppers, *C. geminatus*. Only indefinite particles were observable in the preparations from other viruliferous vectors, *S. acutus* and *S. luteolus*, and from nonviruliferous leafhoppers of the 3 species.

No recognizable symptom of virus infection appeared on the plants used in infectivity tests with the final preparations from the 3 diseased plant materials. These final preparations weakly absorbed ultraviolet light in the region of 260 mμ. The preparations were not chemically tested for protein or analyzed for phosphorus or nitrogen.

DISCUSSION.—The method has 3 salient features: dilutive extraction of aqueous soluble substances from the ground tissues, the clarification of the crude juice by filtration through a Celite cake, and the adsorptive fractionation of amphoteric substances and their subsequent elution with a reduced volume of buffer. Extracts processed through these steps are then suitable for ultracentrifugation, which generally is effective in fractionating particles (of virus size) if

suitable conditions prevail.

The dilutive extraction was necessary to obtain a liquid amenable for clarification, adsorption, and differential sedimentation. Steeping the ground tissues, 200 g, in 2 liters of 0.1 M phosphate buffer at pH 8.5 provided ample solubilization of substances, dispersion of aggregates, and disintegration of coacervates, adsorptive complexes, or loosely bound complexes between substances and fragmented tissues. Dilution of the extractable liquid of the tissues to this extent also reduced the viscosity of the crude extract sufficiently to permit its clarification by filtration.

Clarification of the alkaline extract by filtration through the pores or interstices of the compacted Celite cake removed particles approximately the size of bacteria and larger by physical retention. Smaller particles bearing a negative charge should pass through the pores of the cake; presumably, the cake is negatively charged, since Celite particles are silicates and thus acidic in nature. Positively charged

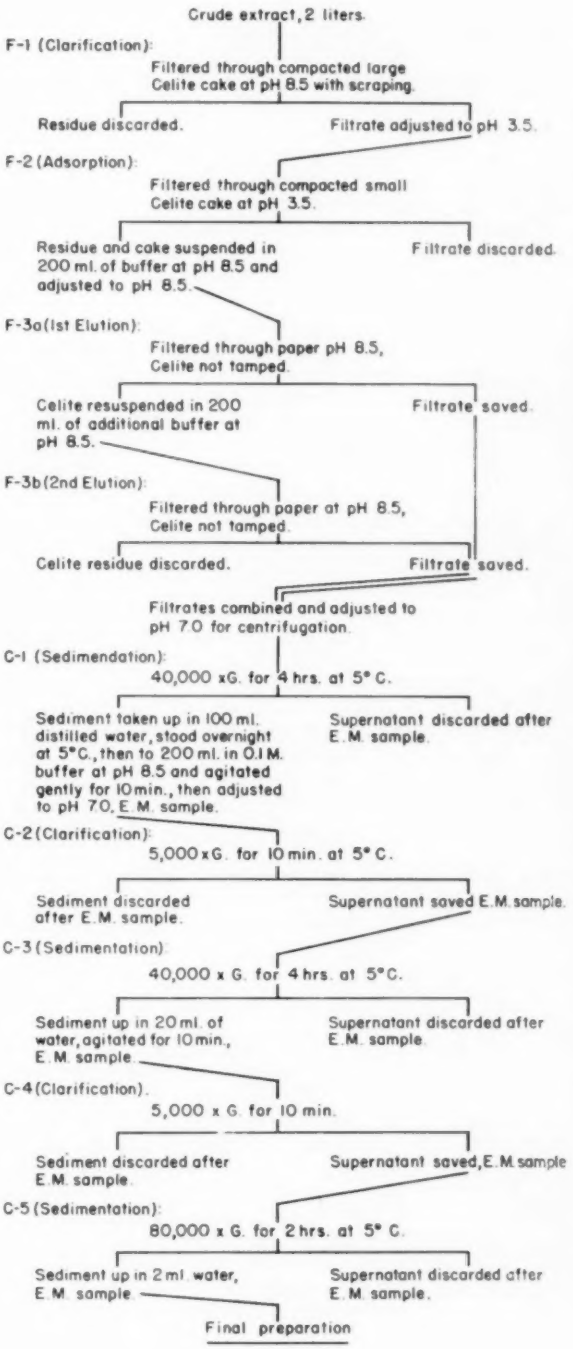
Crude extract, 2 liters.
F-1 (Clarification):
Filtered through compacted large Celite cake at pH 8.5 with scraping.
Residue discarded.
Filtrate adjusted to pH 3.5.
F-2 (Adsorption):
Filtered through compacted small Celite cake at pH 3.5.
Residue and cake suspended in 200 ml. of buffer at pH 8.5 and adjusted to pH 8.5.
Filtrate discarded.
F-3a (1st Elution):
Filtered through paper pH 8.5, Celite not tamped.
Celite resuspended in 200 ml. of additional buffer at pH 8.5.
Filtrate saved.
F-3b (2nd Elution):
Filtered through paper at pH 8.5, Celite not tamped.
Celite residue discarded.
Filtrate saved.
Filtrates combined and adjusted to pH 7.0 for centrifugation.
C-1 (Sedimendation):
40,000 xG. for 4 hrs. at 5° C.
Sediment taken up in 100 ml. distilled water, stood overnight at 5°C., then to 200 ml. in 0.1 M. buffer at pH 8.5 and agitated gently for 10 min., then adjusted to pH 7.0, E. M. sample.
Supernatant discarded after E.M. sample.
C-2 (Clarification):
5,000 x G. for 10 min. at 5° C.
Sediment discarded after E.M. sample.
Supernatant saved E.M. sample.
C-3 (Sedimentation):
40,000 x G. for 4 hrs. at 5°C.
Sediment up in 20 ml. of water, agitated for 10 min., E.M. sample.
Supernatant discarded after E.M. sample.
C-4 (Clarification).
5,000 x G. for 10 min.
Sediment discarded after E.M. sample.
Supernatant saved, E.M. sample
C-5 (Sedimentation):
80,000 x G. for 2 hrs. at 5° C.
Sediment up in 2 ml. water, E.M. sample.
Supernatant discarded after E.M. sample.
Final preparation

FIG. 1. A verbal and graphic flow chart that summarizes the purification procedures involving filtration and centrifugation.

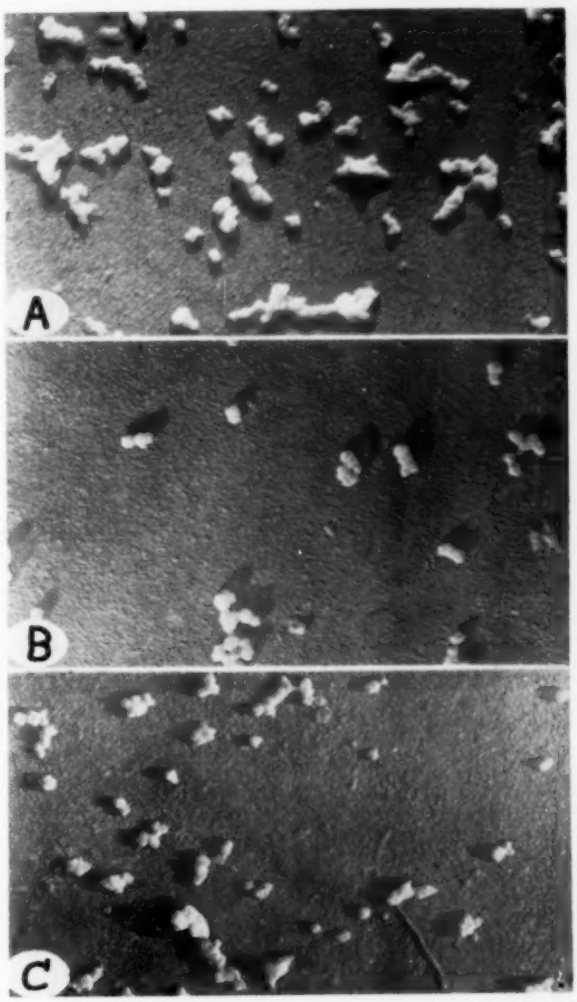

FIG. 2. Electron micrographs of virus-like particles from diseased plants. A) From elm phloem necrosis material. B) From peach X-disease material. C) From cherry ring spot material. Particles of A and B are approximately 50 mμ in diameter, and those of C are approximately 80 mμ in diameter.

particles adsorbable to the negatively charged Celite cake would not pass through the filter. Viruses should appear in the filtrate because they are smaller than bacteria and presumably are negatively charged at pH 8.5, since the plant viruses that have been characterized as nucleoproteins have an isoelectric point far below this pH value.

Adsorption and subsequent elution primarily reduced the volume of the extract, although they also provided some fractionation of small particles. In the adsorptive step at pH 3.5, the filter cake retained amphoteric substances that developed a positively charged surface at this pH or aggregated into nonfilterable particles. This step allowed other substances and the dispersing medium to pass through into the filtrate, which was discarded. Elution in a small amount of buffer at pH 8.5 of the substances retained by the filter cake reduced the volume considerably for subsequent differential sedimentation.

The isolation of virus-like particles from diseased but not from healthy plant materials demonstrates an association of these particles with the diseases. Although the particles are similar in size and shape to some viruses, they cannot be regarded as viruses, since their infectiousness was not demonstrated. The failure to obtain observable infections in the biological assays, however, does not prove that the particles are noninfectious; similar tests with expressed juice from diseased plants have been unsuccessful for these viruses, with the possible exception of cherry ringspot virus.

The finding of 50-mμ particles in preparations from both peach X-disease material and viruliferous vectors of the virus causing this disease but not from the respective controls suggests that these virus-like particles may be the X-disease virus. Certainly the biological differences between plant material and the leafhoppers should be far greater than the differences either between diseased and healthy plant material of the same species or between viruliferous and nonviruliferous leafhoppers of the same species. Failure to find virus-like particles in preparations from other viruliferous vectors may have been due to faulty operations or to a content of virus too low for isolation and detection by the procedures used.

Biological significance of the association of these virus-like particles with the diseases is questionable, since noninfectious particles in this order of size have been isolated from plants with viral infections (4, 5, 6, 9, 13, 16, 17), from plants supposedly healthy (3, 8, 10, 12, 15, 16, 21), and from plants with a disease but from which a causal agent has not been transmitted experimentally (19). In addition, enzymatically active spherical particles in the order of size of viruses have been isolated from presumably healthy plant tissues and cells: 20-mμ particles from tissues of peanut cotyledons (14) and 85-mμ particles from cells of a species of bacterium (7). The plants supposedly healthy may, however, have unrecognized viral infections that are yet undetectable because suitable hosts or transmitting techniques are not known.

The findings support a conclusion that the virus-like particles are indicators of the diseases and thus have diagnostic significance. They also support a provisional method as a guide for isolating viruses from woody plants.

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A NEW PROCEDURE FOR EMBRYO EXAMINATION FOR MYCELIUM OF SMUT FUNGI [1]

W. Popp

SUMMARY

The sectioning instrument used consists of a Spencer rotary microtome fitted with the following: 1) a platform mounted in the knife clamp to support the object being sectioned, 2) a device clamped in the jaws of the microtome head for holding the razor-blade cutting knife, and 3) a dial that fits around the shaft of the crank handle and serves as a thickness gauge. The material is cleared in a solution of 12 per cent ethanol containing 10 per cent potassium hydroxide, washed in water, treated with isopropyl alcohol containing 10 per cent lactic acid, again washed in water, and then embedded in soap on glass slides by means of a glass ring that serves as a mold. Sections are cut singly; after the soap is dissolved in warm distilled water and the water replaced with ethanol, the sections are stained and examined in 45 per cent acetic acid containing 0.2 per cent trypan blue. The fungus stains deep blue, the cell walls of the host stain light blue and the cell contents of the host remain clear. Although devised for rapidly demonstrating the presence of mycelium of *Ustilago tritici* in embryos of wheat grains, the method should be useful for other examinations of the morphology and distribution of fungi in plant tissues.

In preliminary studies of the development of the loose smut fungus (*Ustilago tritici* (Pers.) Rostr.), it was realized that a rapid method was required for demonstrating the presence of the mycelium in infected embryos of wheat grains. Soap,[2] rather than paraffin, appeared promising as an embedding medium; since soap is water soluble, it was thought that little treatment of the tissue before sectioning and staining would be needed. A method satisfactory for these purposes has been devised. This method may also be of use in other situations where rapid preparation of sections is desirable.

APPARATUS.—For sectioning, a Model 820 Spencer rotary microtome is used. It is fitted with the following:

1) A device (Fig. 1, A), made of 0.103-in. sheet aluminum alloy and mounted in the knife clamp (Fig. 2), that serves as a platform to support the object being sectioned.

2) A device (Fig. 1, B), also of 0.103-in. sheet aluminum alloy, that clamps into the jaws of the microtome head (Fig. 2) and to which is bolted the

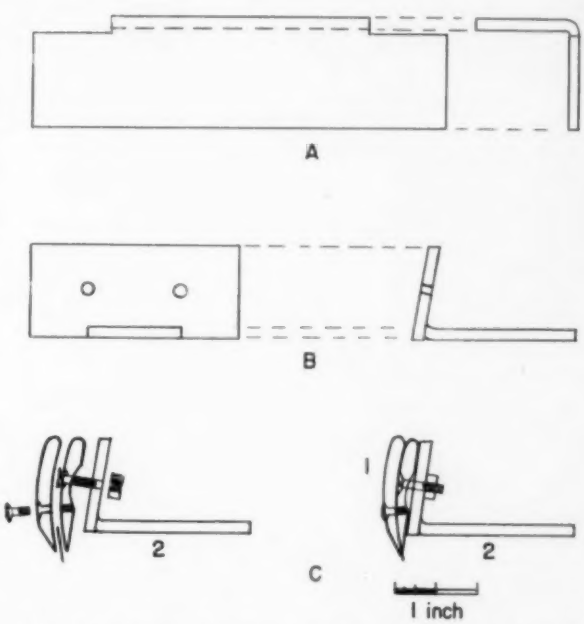

FIG. 1. Microtome fittings. A) Device that is mounted in the knife clamp and serves as a platform to support the object being sectioned. B) Device that is bolted to the cutting knife and clamps in the jaws of the microtome head. C) End view of mounted cutting knife: 1) cutting knife and 2) mounting device.

[1] Accepted for publication July 22, 1957.
Contribution No. 1615 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] McClung, C. E. 1929. Handbook of microscopical technique. Paul B. Hoeber, New York. 495 p.

cutting knife (Fig. 1, C-1) consisting of a razor blade in the special holder designed for the microtome.

3) A circular piece of cardboard, marked off in segments, that fits around the shaft of the crank handle (Fig. 2) and serves as a thickness gauge.

In use, the angle of the cutting knife is adjusted by manipulation of the universal joint of the microtome head. The instrument is operated with the automatic advancing mechanism set to zero. Upward and downward movement of the knife in slicing is controlled by the hand wheel, whereas advance of the knife to the initial and succeeding cutting strokes is effected by the hand crank. As mentioned above, this crank in conjunction with the graduated dial mounted behind it also serves as a thickness gauge. One revolution of the crank, for example, has a calibrated value of 280μ. Fractional turns are calibrated proportionately and may be gauged accurately by referring to the graduations on the dial.

PREPARATION OF TISSUE.—The embryos are extracted by treating the seed with 5 per cent potassium hydroxide.[3, 4] They are then placed in 12 per cent ethanol containing 10 per cent potassium hydroxide and heated in a pressure cooker until they are clear (approximately 30 minutes at a pressure of 5 lb./sq. in.). The embryos are now given a 10-minute washing in warm distilled water and then soaked for 10 minutes in heated isopropyl alcohol containing 10 per cent lactic acid. To aid in orientation they are stained in a 0.1 per cent aqueous solution of trypan blue and then given two 15-minute washings in warm distilled water before being embedded.

INFILTRATION AND EMBEDDING.—Infiltration consists in soaking the embryos 15–30 minutes in a hard soap of high quality that has been melted in hot distilled water to the consistency of melted paraffin. Addition of a little 95 per cent ethanol to the melted soap results in an optically clear embedding medium.

After infiltration, the embryos are embedded by a method that leaves the block firmly fixed to a glass slide. A glass ring is sealed to the surface of the slide by use of Tissuemat (a paraffin compound). A water-soluble glue is then smeared on the floor of the chamber and allowed to dry. The chamber is now half filled with melted soap. After the soap hardens, a drop of melted soap containing the infiltrated embryo is placed on top. The embryo is now oriented and the chamber is filled to the brim with melted soap. On the following day, the glass ring is cut free from the block and removed. The slide and the embedded embryo are then stored until the soap is firm enough to cut.

[3] Popp, W. 1951. Infection in seeds and seedlings of wheat and barley in relation to development of loose smut. Phytopathology 41: 261-275.

[4] Simmonds, P. M. 1946. Detection of the loose smut fungi in embryos of barley and wheat. Sci. Agr. 26: 51-58.

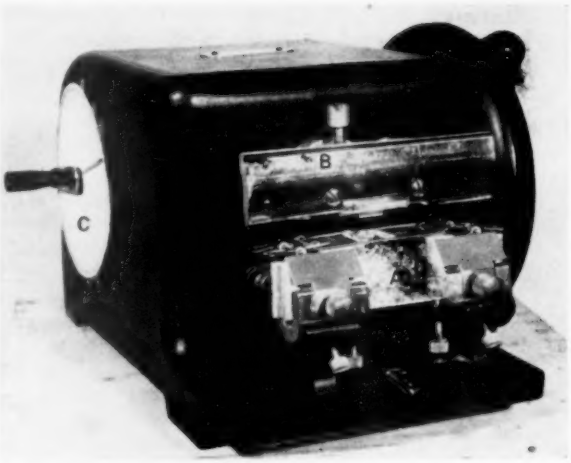

FIG. 2. Modified Spencer rotary microtome. A) Cutting platform mounted in the knife clamp, with a microscope slide bearing the object to be sectioned mounted on top and fastened down with picture-hanger clips. B) Cutting knife attached to mounting device clamped in the jaws of the microtome head. C) Circular piece of cardboard marked off in segments and fitted around the shaft of the crank handle to serve as a thickness gauge.

SECTIONING.—The slide is clamped to the supporting platform. Paper strips are glued to the slide on either side of the block to prevent the knife from striking the slide. The cutting blade is advanced to meet the side of the block facing the operator. Manipulation of the hand crank in a clockwise direction to a predetermined point on the dial now determines the thickness of the section to be cut. Although soap cuts well, thick sections (25–50 μ) are most easily handled and were most suitable for purposes of embryo examination.

STAINING.—The sections are removed from the knife with a dry brush and dropped into a small beaker where the soap is dissolved away in warm distilled water. The sections may then be treated in any required manner. For embryo examination, the section is mounted on a microscope slide and, after a brief treatment with 95 per cent ethanol, stained and examined in 45 per cent acetic or lactic acid containing 0.2 per cent trypan blue.[5] The fungus stains deep blue and the cell walls of the host stain light blue; the cell contents of the host remain clear.

DISCUSSION.—The above method has been used on a variety of diseased tissues (Fig. 3, A, B), and in each instance the presence of the fungus was satisfactorily demonstrated. There are certain limitations to the method, however. Serial sectioning, for example, is tedious, since each section must be handled individually. Some materials may tend to disintegrate after the soap is removed; this can be partially overcome by affixing the sections to a slide and then removing the soap.

[5] Bodijn, K. B. 1956. Trypan blue as a stain for fungi Stain Technol. 31: 115-116.

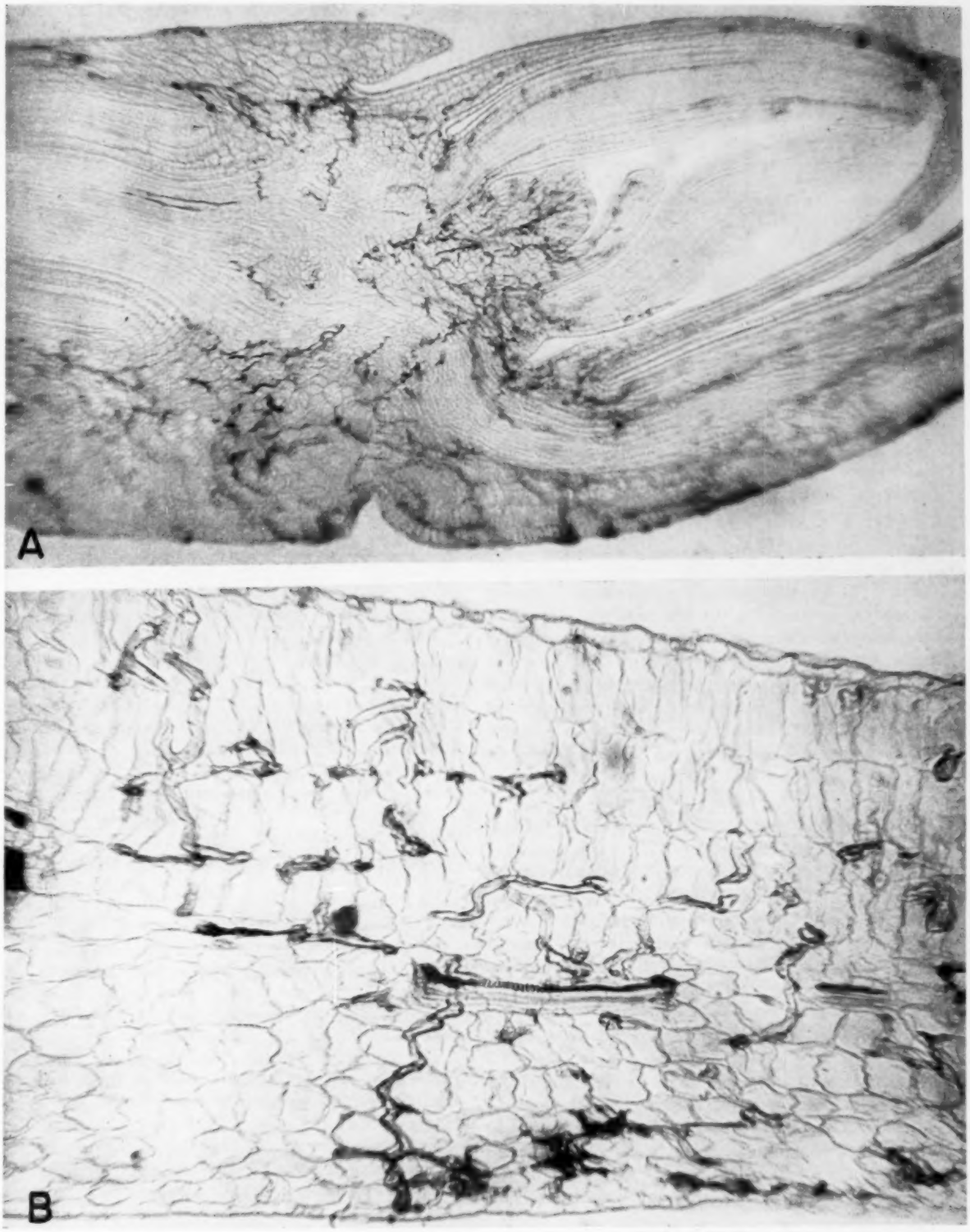

FIG. 3. A) Median longitudinal section of an embryo of a grain of Reward wheat infected with hyphae of *Ustilago tritici* (×114). B) Section of a leaf of sunflower invaded by hyphae of *Sclerotinia sclerotiorum* (Lib.) d By. (×114).

The method works equally well with fresh, dried, or preserved materials, provided that delicate objects are treated in the clearing solution of potassium hydroxide in ethanol only at room temperatures. Compared with the standard paraffin method, less processing is required and the possibility of excessive hardening is avoided. Moreover, the method makes possible an inexpensive and flexible alternative means of sectioning with a rotary microtome. The procedure should be of advantage in any studies that entail routine examinations of the morphology and distribution of fungi in plant tissues.

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ENVIRONMENT AND SORGHUM KERNEL SMUT [1]

C. H. Hsi [2]

SUMMARY

Previous studies have repeatedly indicated the influence of environmental conditions on the infection of sorghum by the covered-kernel smut fungus, *Sphacelotheca sorghi* (Link) Clint., during the pre-emergence period. The relationship between post-emergence environment and infection, however, is yet insufficiently understood. Under drouth conditions in eastern New Mexico in 1953, time of post-emergence irrigation was observed to have affected the amount of infection by this fungus. Early-irrigated plants produced considerably more disease than the late-irrigated ones. Results from experiments in 1954 and 1955 substantiated the 1953 observations. High temperatures following planting resulted in less smut than did moderate temperatures, regardless of soil-moisture conditions existing before or after emergence.

Tapke (8) in 1948 made a comprehensive review of literature pertaining to the environmental influences of the infection of sorghum by the covered-kernel smut fungus, *Sphacelotheca sorghi* (Link) Clint. Low soil temperature and moisture and slightly acid soil during the pre-emergence period were conducive to infection (1, 2, 3, 4, 5, 6, 7). Largely because of these findings, serious consideration has not been given to the post-emergence environmental influences in previous studies.

During the dry summer of 1953, east of Clovis, New Mexico, a field that had been irrigated 2 weeks after the emergence of Redlan sorghum seedlings produced many more smutted heads than did a neighboring field irrigated 2 months after planting. Both fields received exactly the same treatments except for the time of applying irrigation water. This phenomenon led to the conducting of several controlled experiments in 1954 and 1955. Results from these experiments, in addition to the 1953 observations, are contained in this paper.

MATERIALS AND METHODS.—*Experiments in 1954.*—The inoculum was collected from the heavily smutted field in 1953. Chlamydospores from crushed smut balls were thoroughly mixed with the Redlan seed except for the noninfested check. A portion of the artificially inoculated seed was subsequently surface treated with Arasan (50% bis(dimethylthiocarbamoyl) disulfide) and included in the experiments.

Three monthly plantings were made on previously irrigated land; these were made on May 1, June 3, and July 2, 1954. Each planting was a separate experiment and consisted of 4 rows of inoculated seed, 2 rows of inoculated and Arasan-treated seed, and 1 row of noninoculated seed.

Of each monthly planting, 2 rows of inoculated seed, 1 of inoculated and Arasan-treated seed, and 1 of noninoculated seed were irrigated 2 weeks after the seedlings emerged, whereas the remaining rows did not receive any irrigation water until 6 weeks later.

Smut counts were made and rechecked by the same or different individuals after sorghum heading and grain formation in all rows were completed. The dates of heading for plantings made on May 1, June 3, and July 2 and irrigated 2 or 8 weeks after emergence were July 24 and August 12, August 20 and 30, and September 16 and 24, respectively.

The amount of infection was expressed in terms of percentage, as calculated by dividing the number of smutted plants in each row by the total number of plants in the same row.

Experiments in 1955.—Materials and methods used in this year's experiments were similar to those used in 1954. One more postemergence irrigation treatment (5 weeks) was added. Four separate plantings were made on previously irrigated land on May 5, May 14, June 4, and July 1, 1955. Each date of planting was a separate experiment involving a 3 × 3 Latin square design.

[1] Accepted for publication July 24, 1957.
Journal Series No. 112, Agricultural Experiment Station, New Mexico College of Agriculture and Mechanic Arts.
[2] Assistant Plant Pathologist, New Mexico Agricultural Experiment Station.

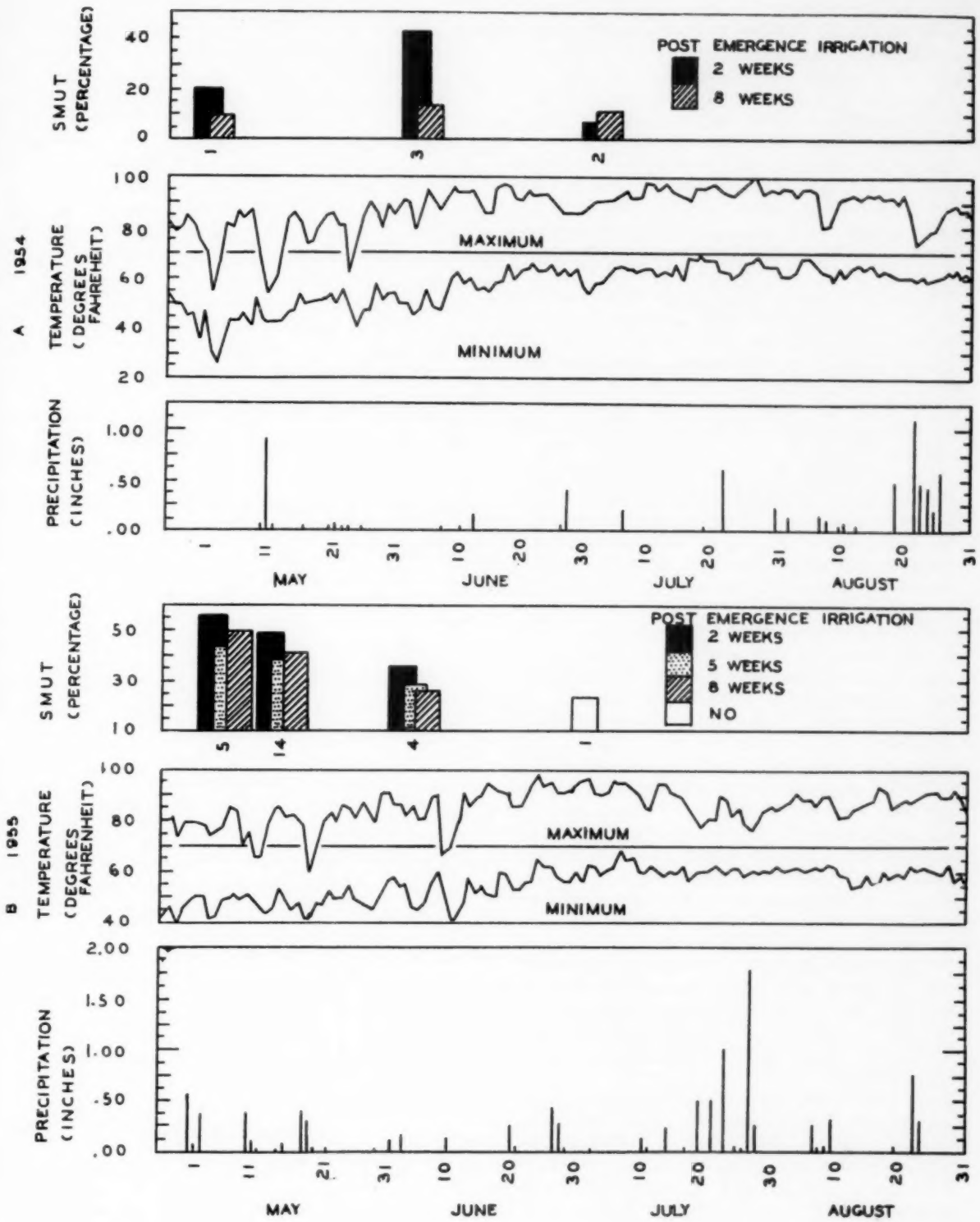

FIG. 1. The influence of precipitation, temperatures, and time of postemergence irrigation upon sorghum kernel smut in eastern New Mexico in A) 1954 and in B) 1955.

RESULTS.—*Farmer's field (1953).*—Around 55 per cent of the total heads were smutted in the field that was irrigated 2 weeks after the emergence of sorghum seedlings. A wide variety of smut symptoms was observed, ranging from heads with a few smutted kernels to completely smutted heads.

Less than 4 per cent of the plants in the neighboring field that was irrigated 2 months after planting (roughly 8 weeks after emergence) were smutted. Though planted at the same time as those in the other field, sorghum plants in this field were nearly 3 weeks later in development than were those in the field irrigated earlier. Total precipitation from date of emergence to postemergence irrigation amounted to less than 1 in.

In the late-irrigated field, smutted heads were found mostly on young tillers that grew after irrigation. Healthy heads on the main stalks, together with smutted ones on the new branches of the same plants, were not uncommon. The smut symptoms were generally clear-cut: either the heads were completely smutted or they were visibly healthy. There were few intermediates.

Experiments in 1954.—When only the dates of planting are considered, infection of the May 1 planting was higher than that of the July 2 planting but lower than that of the June 3 (Fig. 1, A). High temperatures following the July planting apparently caused the significantly smaller amount of infection in the July 2 planting.

With the exception of the July planting, plants produced from inoculated seed and irrigated 2 weeks after emergence had considerably more disease than did those irrigated 8 weeks after emergence. These differences were statistically significant at the 5 per cent level.

No kernel smut was found in rows planted either with noninoculated seed or with seed that was inoculated and treated with Arasan.

The results of the 1954 experiments indicated that early postemergence irrigation under drouth conditions had definitely favored infection by the smut fungus. High temperatures following planting significantly inhibited infection, regardless of soil-moisture conditions existing before or after emergence.

Experiments in 1955.—The rainfall distribution in the summer of 1955 largely masked the effect of different postemergence irrigation treatments. Heavy rainfall after the July 1 planting made this planting useless for comparing differences between the irrigation treatments.

As shown in Figure 1, B, early-planted sorghum had significantly larger amounts of kernel smut than did that planted later in the season. Differences in the amount of infection between various postemergence irrigation treatments within the same date of planting were not statistically significant; however, rows irrigated 2 weeks after emergence consistently contained higher percentages of smutted plants than did the late-irrigated rows. Irrigation at 5 weeks (compared with 8 weeks) after emergence produced only slight differences in the total amount of kernel smut.

DISCUSSION.—Sorghum covered-kernel smut is one of the seedling smuts, systemic in nature. It is not surprising, therefore, that any changes in the environmental conditions prior to the establishment of chlamydospores in the young florets of the host plants directly affect the ultimate phenotypic symptoms of infection. The probable reason that postemergence conditions are yet to receive proper attention may be that any conditions unfavorable for infection by the smut fungus usually inhibit sorghum heading or grain formation. Only under irrigated conditions in a dry season does deficient soil moisture during the postemergence period become evident as a limiting factor in infection.

In the late-irrigated field in 1953, heads of the main stalks escaped smut, whereas those of the young branches (growing after irrigation) of the same plants did not. Differences between the growth rate of the host tissues and that of the fungus mycelium (after the resumption of growth made possible through the availability of water) may have accounted for such differences in infection on the same plants.

Relatively little attention in recent years has been given to the environmental influences on infection by the sorghum kernel smut fungus. This may mean that such information is relatively plentiful and that the disease can be successfully controlled by chemical seed treatments. With the continuous expansion of effective seed treatment both here and abroad and with the possible low yield because of delayed maturity, the use of delayed postemergence irrigation under dry conditions as a means of controlling kernel smut is both impractical and unnecessary.

When heavy infection is desired, such as in the disease study or in a breeding program for smut resistance, early postemergence irrigation may be used for the purposes of obtaining high percentages of smutted plants or for increasing the certainty of obtaining an epidemic, if due considerations are given to the planting date, soil moisture content, and soil temperatures. Changes in soil moisture or other conditions during the postemergence period may be helpful in our understanding of the complete developmental process of systemic plant diseases other than covered-kernel smut of sorghum.

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PLAINS SUBSTATION
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THE EFFECT OF ENVIRONMENTAL FACTORS AND PLANT AGE ON THE INFECTION OF RICE BY THE BLAST FUNGUS, PIRICULARIA ORYZAE[1]

Robert P. Kahn and John L. Libby

SUMMARY

A quantitative bioassay was utilized to assess the effects of plant age and various environmental factors on the infection of Caloro and Zenith rice leaf blades by *Piricularia oryzae*. Infection occurred between 60° and 95°F in dew cabinets with 16–20 hours of dew exposure; the optimum was 80° or 85°. The minimal periods required to initiate infection under dew-cabinet conditions were 10 hours at 80°, 12 hours at 70°, and 14 hours at 65°. The upper surfaces of leaf blades were twice as susceptible as the lower surfaces. With increasing age of plant, leaves, or portions of the leaf blade, there was progressive decrease in susceptibility to infection. On susceptible varieties, leaves older than 8–10 weeks were resistant. Leaves of susceptible plants grown on non-flooded soils were more susceptible to infection than were leaves of plants grown on flooded soil. Greenhouse-grown plants were more susceptible than field-grown plants of the same age.

Although the blast disease of rice, caused by *Piricularia oryzae* Cav., has usually been considered of minor importance in the United States, severe epiphytotics have occurred recently at Belle Glade, Florida,[2] and in Louisiana (6). Hence, the blast disease may represent a potential threat to rice in the warmer and more humid areas of the United States.

Despite the extensive literature dealing with the effect of environmental factors on infection, there appear to have been no critical studies in which only 1 factor was varied while others were held constant.

The investigations reported herein were undertaken to provide basic information concerning the influence of environmental and other factors on the infection of rice. The quantitative procedures and equipment developed in this laboratory (7, 16, 17) were utilized. It is conceivable that such information in conjunction with an analysis of climatological data from the rice-growing areas of the United States could assist in predicting where and under what conditions blast might represent a threat to the rice crop. This type of information might be useful also in suggesting cultural practices that would reduce the severity of blast epiphytotics.

[1] Accepted for publication July 26, 1957.
[2] Communication from Dr. Paul R. Miller, Agricultural Research Service, Beltsville, Maryland.

REVIEW OF LITERATURE.—Previous investigations have utilized natural epiphytotics or artificial spray-inoculation procedures in studies on the effect on infection of plant age, nutrition, cultural practices, and environmental factors. It has been reported that leaves of seedling and early-tillering plants are more susceptible to infection than are those of older plants (2, 5, 8, 9, 22, 23, 25) and that leaves of plants grown in dry soil are more susceptible than those of plants grown in flooded soil (9, 10, 15, 20, 26, 27). In some of these investigations, plant age or rice cultural methods were related to various morphologic characteristics, such as relative number of stomates or thickness of the epidermal layer, in an attempt to differentiate between susceptible and resistant varieties. Studies of the silicon content of the plant or its parts (3, 12, 13, 18, 24, 26, 27, 28, 29, 30, 31) and the availability of nitrogen in the soil (3, 9, 19, 26, 27, 31) have led to the general, though not unanimous, conclusion that high silica content and/or low nitrogen availability are associated with resistance.

The influence of temperature and moisture on infection also has been considered by some investigators. Hemmi and Abe (11) found that an air temperature of 72°–82°F favored high infection and that the minimal exposure to moisture required to initiate infection at these temperatures was 6–8 hours. Anderson, Henry, and Tullis (5) reported that infection was

TABLE 1.—*Culture numbers, physiologic race numbers, and varieties of rice used*

Race[a]	Culture number	Test variety[b]
1	635	Zenith
2	603	Caloro
3	640	Caloro
4	776	Caloro
6	747	Caloro
7	765	Caloro or Zenith
8	770	Caloro
9	927	Caloro
11	923	Caloro

[a] Races 1 and 2 described by Latterell et al (14). The other races were also identified by Latterell and coworkers (unpublished data).

[b] Zenith and Caloro mature in approximately 120 and 130 days, respectively, during summer months in the greenhouse in Maryland.

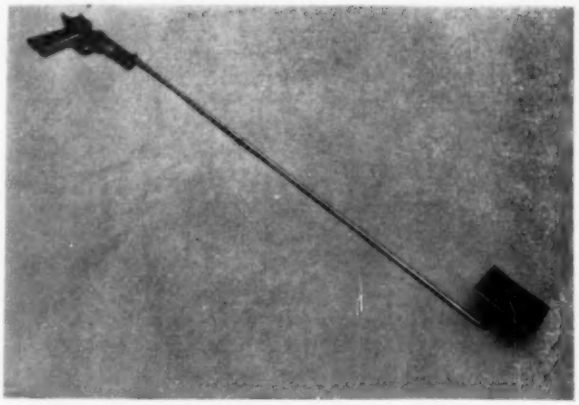


FIG. 2. CO_2 pistol, showing muzzle extension designed to direct the discharge through a hole in the center of the baffle plate. (U. S. Army Photograph.)

maximal between 72° and 82° at 16–24 hours of exposure and that infection did not occur on leaves exposed for less than 10 hours. Hashioka (9) observed that temperatures between 68° and 90° were conducive to infection but that the optimum was between 79° and 82°. Nishikado (21) reported that the optimal temperature for the growth of the fungus on agar was between 80° and 84°.

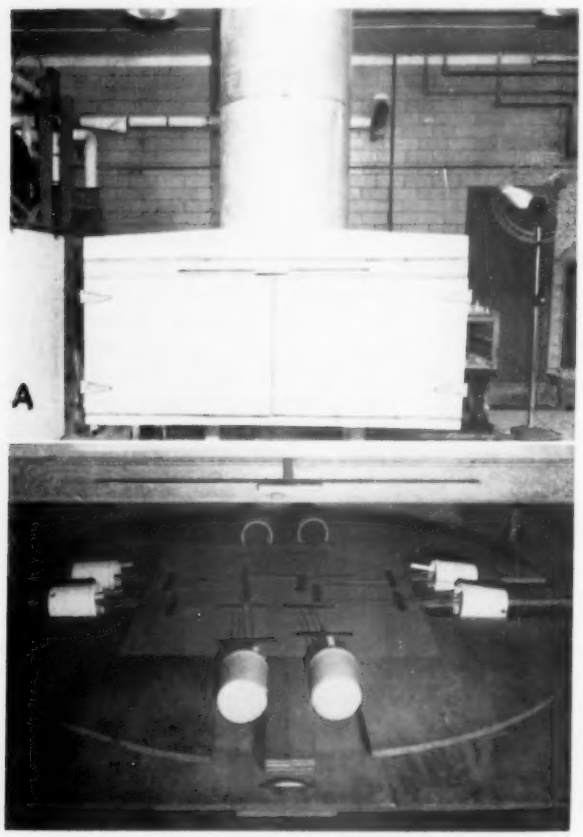


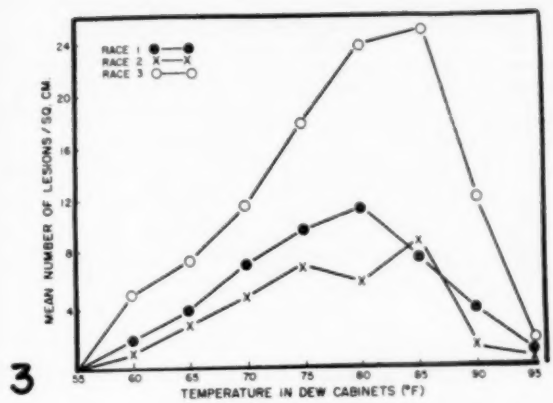
FIG. 1. Apparatus employed to effect uniform spore depositions. A) Exterior view of settling tower, showing base cabinet, baffle plate, and cylinder. B) Turntable, showing a portion of the plants in pots located in tapering slots. In actual use, the turntable is centered under the cylinder. (U. S. Army Photographs.)

MATERIALS AND METHODS.—*Isolates of the blast fungus and varieties of rice.*—Several isolates of *Piricularia oryzae* were utilized. The culture numbers and race identifications of the isolates and test varieties of rice are shown in Table 1.

Rice culture.—Plants were grown in ¼-gal. pots under greenhouse and field conditions on Duffield silt loam either in flooded or nonflooded culture. Flooded conditions were provided by maintaining the water level 3 to 6 in. above the soil in pots located in tank-type benches in the greenhouse or in trays (2 ft. × 8 ft. × 10 in. high) in the field. The water was changed 4 to 6 times per month. Plants were fertilized with soluble 15–30–15 fertilizer at 30-day intervals beginning at a plant age of 6 weeks.

Bioassay.—Inoculations were performed by exposing leaf laminae to uniform spore deposition in a modified form of the settling tower (Fig. 1, A) described by Bell et al (7) and Miller (16).[3] The inoculation apparatus consisted of a wooden base cabinet (6 × 6 × 3 ft. high) housing a turntable and supporting a metal cylinder (5 ft. high and 2½ ft. in diameter). The turntable (5 ft. in diameter) contained at the periphery 8 tapering slots to accommodate 8 pots of plants in a horizontal position and to orient the leaf blades under the center of the cylinder (Fig. 1, B). The leaves were held flat by metal weights. Dry spores (usually 20 mg) were discharged into the tower by a CO_2 pistol (Fig. 2) provided with an adapter that directed the blast vertically upward through a small hole in a baffle plate between

[3] The authors wish to acknowledge the assistance of Dr. R. D. Schein in standardizing the quantitative bioassay employed in these investigations.



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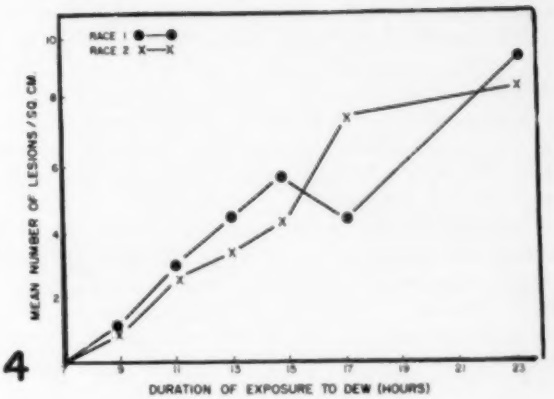

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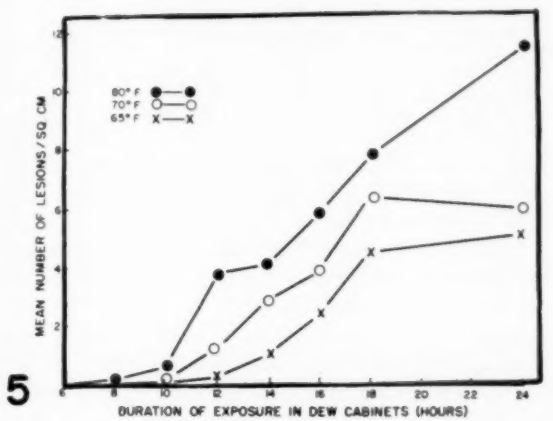

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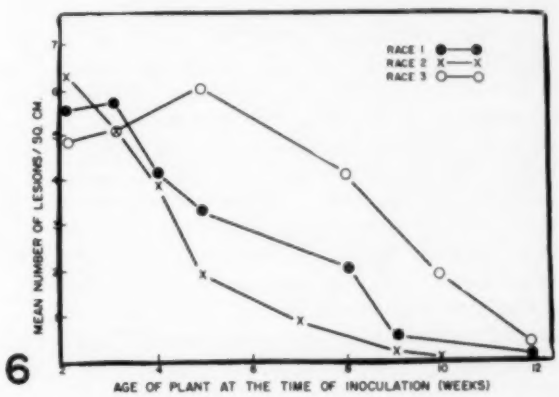

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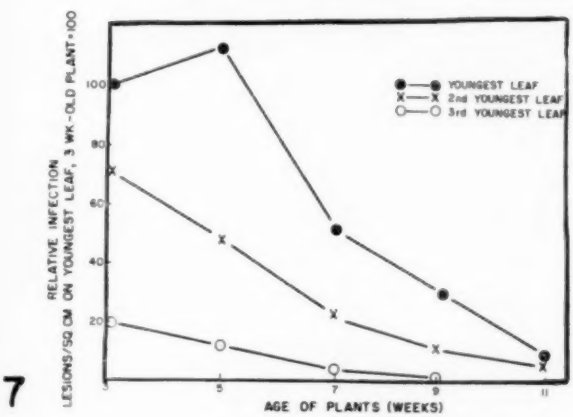

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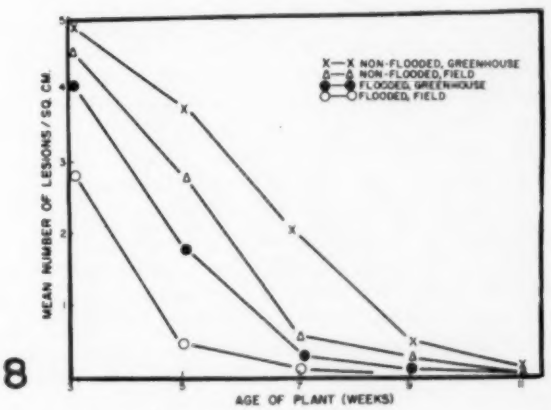

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Fig. 3–8. Number of eyespot lesions as influenced by various factors during the infection of Caloro or Zenith rice by the blast fungus.—Fig. 3. Influence of temperature. Sixteen- to 20-hour exposures with Races 1, 2, and 3 on the youngest leaf of 4- to 5-week-old nonflooded greenhouse-grown plants.—Fig. 4. Influence of dew periods at 80° F. Races 1, 2, and 3 on the youngest leaf of 4- to 5-week-old nonflooded greenhouse-grown plants.—Fig. 5. Influence of dew period at 65°, 70°, and 80°F. Race 2 on the youngest leaf of 4- to 5-week-old nonflooded greenhouse-grown plants. —Fig. 6. Influence of plant age. Races 1, 2, and 3 on the youngest leaf of nonflooded greenhouse-grown plants; 16- to 20-hour dew-cabinet exposure at 80°.—Fig. 7. Influence of leaf age. Nonflooded greenhouse-grown plants at 3–11 weeks of age. (Mean lesion counts of Races 1, 2, 3, and 7).—Fig. 8. Influence of rice cultural methods. The youngest leaf on nonflooded and flooded greenhouse-grown plants and on nonflooded and flooded field-grown plants between 2 and 13 weeks of age. (Mean of Races 4, 6, 7, 8, 9, and 11.)

the turntable and the cylinder. The movable baffle was maintained in position for 1 minute after discharge to intercept any spore clumps that might be present. The plate was then withdrawn and the leaves exposed to the spore shower for 11 minutes, after which the test plants were transferred to dew cabinets (17). Following the experimental exposures in the dew cabinets, the plants were transferred to the greenhouse. At the end of 4–6 days, lesions were counted on 10 cm of leaf blade, and these counts were converted to an area basis in order to evaluate the various experimental treatments.

RESULTS.—*Influence of temperature.*—The temperature in the dew cabinet during the first 16 to 20 hours of the infection process influenced the relative number of eyespot lesions (Fig. 3). The results with 3 races showed that infection that would subsequently develop into eyespot lesions occurred over the range 60° to 95°F. Maximal infection occurred at 80° or 85°.

Influence of dew period.—The duration of exposure to dew at 80°F influenced also the number of infections that subsequently developed into eyespot lesions. The minimal period of exposure required for infection at 80° was 9 or 10 hours (Fig. 4, 5). Infection increased with increased length of exposure to moisture up to 23–24 hours. At temperatures below 80°, periods longer than 10 hours were required for initiation of infection and the level of infection was lower (Fig. 5) than at 80°. After a 24-hour dew period, there were twice as many lesions on plants subjected to the 80° dew-cabinet incubation as on plants at 70° or 65°F.

Upper and lower surfaces of leaf blades.—The upper surfaces of the youngest leaves on 3- and 4-week-old Zenith or Caloro plants were approximately twice as susceptible to infection by Races 1, 2, or 3 as were the lower surfaces (Table 2).

Age of plant.—Susceptibility of leaf blades to infection decreased with increased age of the plant (Fig. 6). During the winter months on nonflooded soil, susceptibility of the youngest leaf was maximal in 1- to 4-week-old plants, and it decreased with increased plant age up to 10 or 11 weeks. Leaves of plants 11–24 weeks of age were resistant. Leaves on plants grown in the greenhouse during summer months became resistant at approximately 8–9 weeks.

Age of leaves.—On plants of any given age up to approximately 9–11 weeks, the youngest or uppermost leaf was more susceptible than the next older one, which in turn was more susceptible than the next older (Fig. 7). With increasing plant age, the susceptibility of each of the 3 youngest or uppermost leaves decreased progressively. The same results were obtained in experiments where the actual age of each leaf in days was known as reported in other investigations from this laboratory (30).

Region of the leaf blade.—Differences in the susceptibility of different portions of the same leaf blade are demonstrated in Table 3. Prior to inoculation, approximately two thirds of each blade was masked with Kraft paper, and the remaining third was exposed to the spore shower. Lesion counts were made for the central 5-cm portion of each region. The basal third of the blade averaged significantly more lesions than did the central or apical thirds. Thus, the most resistant area was the apical portion, which is the oldest portion of the leaf.

TABLE 2.—*Susceptibility of upper and lower surfaces of leaf blades of greenhouse-grown Caloro or Zenith rice to the blast fungus*

Race of pathogen	Rice variety	Age of plant at the time of inoculation (weeks)	Mean number of lesions/cm²	
			Upper surface	Lower surface
1	Zenith	3	5.6	2.4
		4	5.6	2.8
2	Caloro	3	2.0	1.2
		4	4.0	1.2
3	Caloro	3	28.0	12.8
		4	14.4	4.4
Mean			9.9	4.1

Methods of rice culture.—Decreased infection with increased age was obtained with greenhouse-grown plants on flooded and nonflooded soils and with field-grown plants on flooded or nonflooded soils (Figs. 7, 8); however, the level of infection differed with the type of culture. In general, leaves of plants grown in flooded soil were more susceptible than leaves of plants grown on nonflooded soil. This observation is in agreement with reports in the literature. Similarly, greenhouse-grown plants were more susceptible

TABLE 3.—*Comparative susceptibility to infection by the blast fungus of basal, central, and apical portions of the youngest leaf blade on nonflooded greenhouse-grown 4- to 5-week-old rice plants*

Leaf blade area[a]		Mean number of lesions/cm² when fungus race and rice variety were as indicated		
		Race 1[b] (Zenith)	Race 2[b] (Caloro)	Race 3[c] (Caloro)
Basal		56	24	64
Central		45	18	68
Apical		19	12	52
LSD[d]	5%	19.9	11.2	4.6
	1%	29.0	16.3	6.7

[a] Rice leaf blades approximately 30 cm long were divided into thirds by an appropriate masking procedure. Lesion counts were obtained from the central 5-cm portions of each region and converted to an area basis.

[b] Means of 56 leaves (8 leaves inoculated on each of 7 days).

[c] Means of 24 leaves (8 leaves inoculated on each of 3 days).

[d] Based on sub-plot error variance derived from a split-plot analysis of variance, in which leaf areas constituted sub-plots and experiments conducted on different days were the main plots.

to infection than were field-grown plants of the same age. Differences in the cultural methods and between greenhouse and field temperatures, however, may have altered growth rates so that plants of the same chronologic age were actually at somewhat different developmental stages.

DISCUSSION.—A decrease in susceptibility with increased age was demonstrated with several races of *Piricularia oryzae* on Zenith and Caloro rice for plants, leaves, and leaf blade regions of different ages. A preliminary survey by the authors shows that this response was also characteristic of several other race-variety combinations.[4] Although the age response may be characteristic of rice varieties in general, the sampling conducted by the authors was too small to preclude the possibility that there may be exceptions among the hundreds of named rice varieties.

The results with greenhouse-grown plants suggest that exposures to moist conditions of more than 10 hours at night temperatures above 70°F are favorable for the infection of leaf blades of susceptible varieties less than 8–10 weeks old. It is interesting to note that severe epiphytotics of blast in June-planted rice occurred in Belle Glade, Florida (1953–1956) during July and August[2] when the night temperatures averaged approximately 70° and the dew periods 11–13 hours. In general, the April-planted rice escaped infection, probably because of the low night-temperatures during most of the period of leaf susceptibility.

Control measures based on cultural practices are suggested by the results herein reported. It is recommended in those U. S. rice-growing areas where favorable environmental conditions for blast occur during the summer that all rice in the area be planted as early in the growing season as possible. This early planting procedure would insure that the period of maximal leaf susceptibility would occur during a period when environmental conditions were not conducive to infection. Thus, the number of lesions on leaves, and hence the sporulating area, should be much smaller than on late-planted crops. The net effect would be to prevent or delay the increase of inoculum and thus minimize the severity of any subsequent infection during the heading stage.

CHEMICAL CORPS
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PATHOGENESIS OF ROOT-KNOT NEMATODES TO THE PORTO RICO VARIETY OF SWEETPOTATO[1]

L. R. Krusberg and L. W. Nielsen

SUMMARY

Porto Rico sweetpotatoes were planted in soil naturally infested with *Meloidogyne incognita acrita* to study the pathogenesis of this nematode in sweetpotato. An inoculum differential was established by applying D-D impregnated on vermiculite in the row to one half of the randomized plots. The influence of the pathogen on plant growth and the histopathological changes in the root system were studied by harvesting and examining plants from replicated plots at 2-week intervals after planting. Three major infection courts were evident, i.e. young root tips, lateral root ruptures, and the surfaces of cracks. After penetration, the second-stage larvae migrated intercellularly and intracellularly to the site of feeding and of subsequent nematode growth. The site of feeding varied with the infection court. Larvae came to rest primarily in the stele in the region of cell elongation, in the cambial zone, or in the parenchyma, depending upon whether the infection court was young root tips, lateral root ruptures, or crack surfaces, respectively. Nematode feeding stimulated the formation of several atypical tissues: giant cells, "abnormal xylem," hyperplastic parenchyma, and cork Giant cells were initiated first. Cork formed about mature females and their egg masses and was most abundant in roots from late harvests. In roots undergoing secondary growth, constrictions developed at sites of numerous infections, particularly around lateral roots. Root cracking was also correlated with these lateral root infections, and cracked roots were numerous in late harvests. Young plants heavily infected had small roots and tops; the roots had numerous cracks.

Root-knot nematodes (*Meloidogyne* spp.) infect sweetpotato (*Ipomoea batatas* (L.) Lam.), but little is known of their injury to the growing plant. In recent years, root cracking has been associated with nematode infection. Root or "growth" cracking is also associated with host nutrition and soil moisture relationships. The objectives of this investigation were to study the role of root-knot nematodes in plant and root development and the histopathology of pathogenesis and root cracking in Porto Rico sweetpotato.

Most reports on nematode injury to sweetpotato are based on field observations. Elliot (6) and Poole and

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Schmidt (14) observed independently that sweetpotato yields were curtailed by root-knot nematodes. Nielsen and Sasser (12) obtained greater yields in infested sandy and loamy soils treated with 1,2-dibromoethane or with D-D (1,3-dichloropropene; 1,2-dichloropropane) than in nontreated soil. Weimer and Harter (18) found that nematodes caused considerable loss of sprouts in seedbeds, but that losses in the field were not serious. Scott (16) and Ogle (13) suggested that nematodes may contribute to root cracking. Mullin (10, 11) was able partially to control cracking by fumigating soil heavily infested with root-knot nematodes, and this was confirmed by Nielsen and Sasser (12). Several workers reported differences in varietal susceptibility to root-knot nematodes (4, 8, 14, 17, 18). The Porto Rico variety is rated fairly resistant to the organism (14). Shibuya (17) found that root-knot larvae entered root tips of both resistant and susceptible sweetpotato varieties in large numbers.

MATERIALS AND METHODS.—A field of sandy loam heavily and uniformly infested with *Meloidogyne incognita acrita* Chitwood 1949 was selected for study. Sweetpotatoes grown in this field the previous year developed severe symptoms of root-knot nematode injury, and assays of scattered soil samples prior to fumigation for the present study averaged 535 larvae per pint. A portion of the infested area was divided into 4 consecutive 50-ft. blocks separated by 15-ft. alleyways. To establish an inoculum differential in the soil, 1 row of each pair, randomly selected, was treated with D-D (in the row at 10 gal. per acre) impregnated on vermiculite (12). All plots were set with Porto Rico sprouts approximately 12 in. apart in the row 24 days after fumigation.

Nematode populations, root infection, and disease development were followed by harvesting at regular intervals starting 18 days after planting. Plants in 1 randomly selected pair of rows from each block were dug and transferred to the laboratory for examination. A composite 1-pint soil sample for nematode assay also was removed from each plot before harvest. Each soil sample was screened by the method of Christie and Perry (3) for all parasitic nematodes. Larval counts of *M. incognita acrita* were transformed to logarithms for statistical analysis. The interval between successive harvests was 14 days except between the last 2 (Sept. 3 and 29), which was 4 weeks.

Several categories of data were recorded from the harvested plants. The root system of each plant was severed, washed, and rated for root knot on the basis of 0 = no galls, 1 = a trace of galling, 2 = scattered galling, 3 = moderate galling, and 4 = severe galling (Fig. 1, A). Tops and roots of each plant were weighed and the enlarging roots counted. The number of cracks per root system was recorded for the last 4 harvests and the number of cracked roots for the

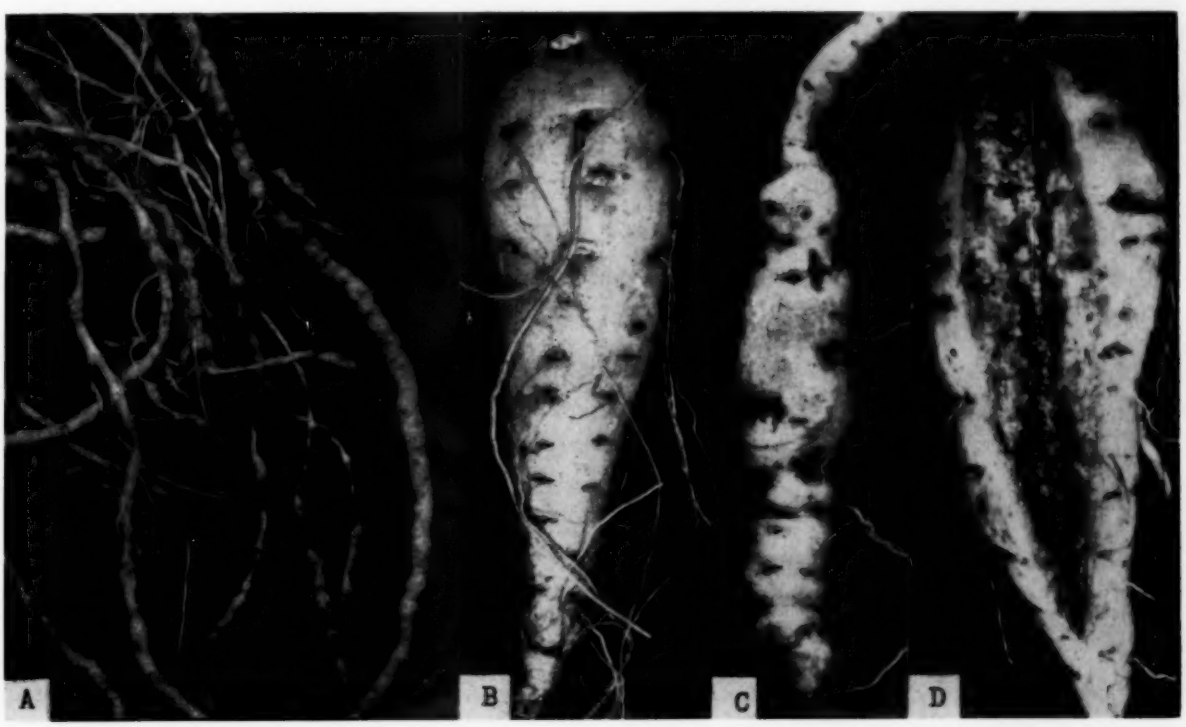

FIG. 1. Morphology of root-knot-affected sweetpotato roots. A) Galling in primary and in enlarging roots 4 weeks after planting. B, C, D) Enlarged roots from fifth harvest. B) No cracks. C) Small cracks. D) Large cracks.

last 2 harvests. The relative yield of U. S. No. 1 potatoes was recorded from the last harvest. At each harvest, data recorded from individual plants in each treatment and replication were summed and the averages subjected to various analyses. Fresh top and root weights also were transformed to logarithms before analysis.

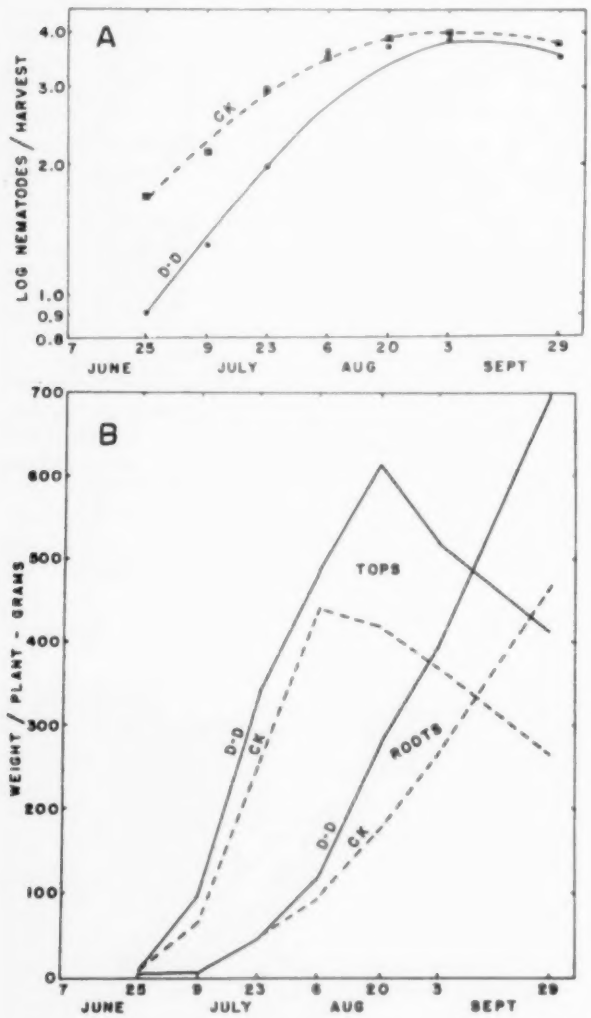

FIG. 2. A) Populations of root-knot larvae in fumigated and nonfumigated soil in the presence of sweetpotatoes. B) Average root and vine (tops) weights of sweetpotato plants grown in fumigated and in nonfumigated soil.

Nematode penetration and histological changes within invaded roots were observed in root samples collected from each harvest. Root-knot indices were the basis of selecting samples for the first 3 harvests and root cracking for the remaining 4 harvests. Examinations were of 2 types: 1) gross examination of tissue involving relatively thick tissue slices in which the positions, number, and stage of nematode development were recorded, and 2) detailed histological examination of paraffin-embedded roots sectioned with a microtome.

Tissue sampling for gross examination was begun at the third harvest where enlarging roots were sampled from root systems with indices of 0, 2, and 4. In the fourth and subsequent harvests, tissue samples were taken from roots with no cracks, with small cracks, and with large cracks (Fig. 1, B, C, D). At each harvest, 25 roots from each of the 3 categories of root-knot index or of cracking were selected, and from each root a piece of tissue $1 \times \frac{3}{4} \times \frac{3}{4}$ in. that included periderm and cracks when present was excised from the side of the potato. Every piece was sliced transversely with a razor into 40–50 slices approximately 500 μ thick and serially threaded on a wire to keep them in natural order. Slices were killed and fixed for 24 hours in CRAF III (30 ml 1 per cent chromic acid, 20 ml 10 per cent acetic acid, 10 ml 40 per cent formalin, 40 ml water) (15) and then placed in 10 per cent ethanol containing acid fuchsin (2 ml of a 1 per cent solution of acid fuchsin per 100 ml of 10 per cent ethanol) for 3 hours at 40°C. The slices of tissue were dehydrated and cleared by a short ethanol-xylol series and individually examined, while mounted in xylol, with a stereoscopic microscope.

Material for detailed histological examination was obtained from roots at all 7 harvests. In addition, vine cuttings were planted in pots of field-infested soil in the greenhouse to obtain material for initial infection. Root tips from these vine cuttings were harvested 3, 4, 5, and 7 days after planting. Five small root pieces from each of the 6 categories used for gross examination plus root tips from vine cuttings were killed, fixed, and prepared for sectioning as described above, except that a slower ethanol-xylol dehydration series

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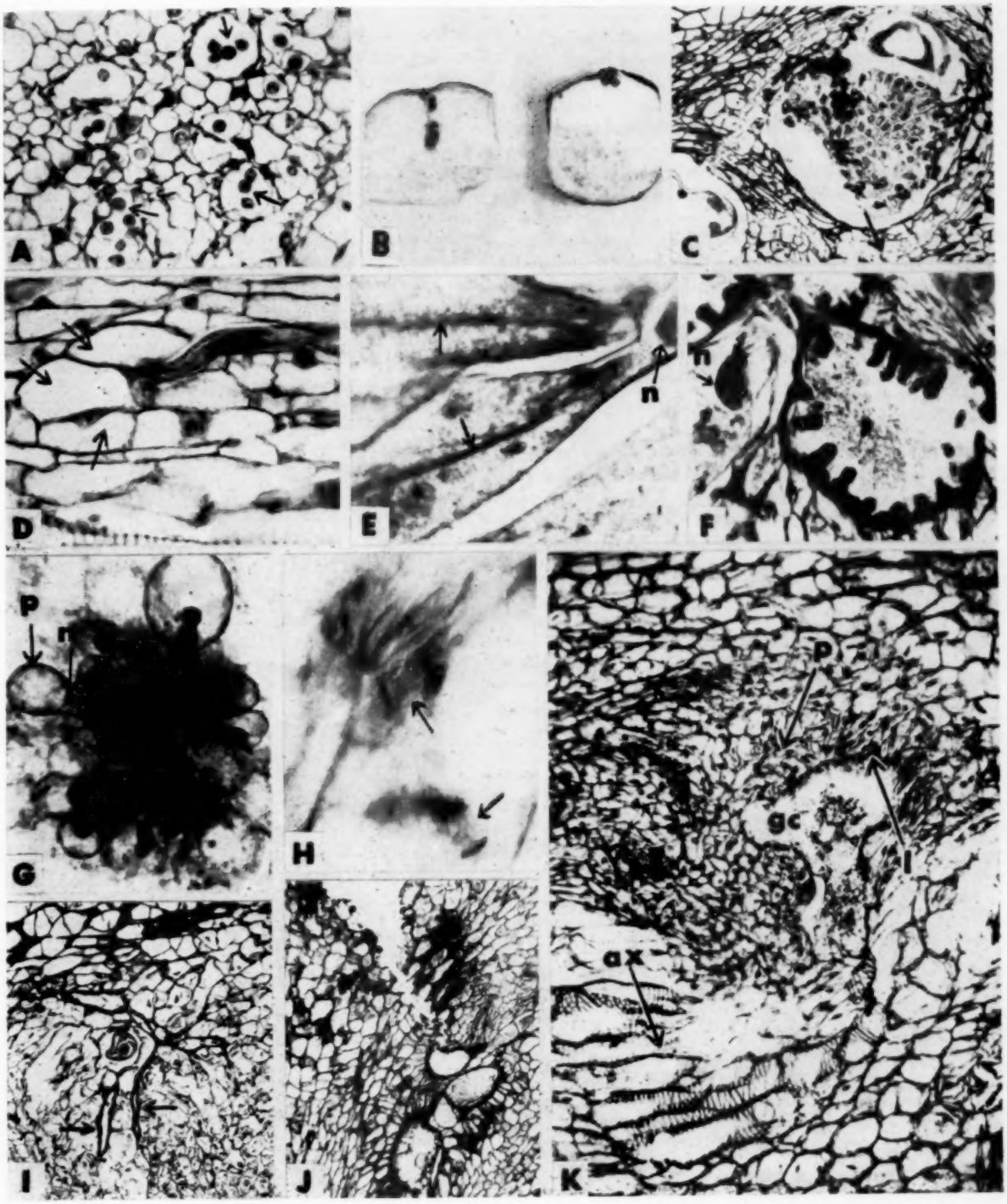
FIG. 3. Histopathology of sweetpotato roots infected by root-knot nematodes. A) Cross section of infected root tip from a 5-day-old vine cutting, showing second-stage larvae (arrows) in center of the root (×220). B) Root cross sections with mature nematodes within lateral root traces. C) Cork formation (arrow) around nematode and egg mass from seventh harvest (×50). D) Initiation of giant cells (arrows) around head of larva in root tip from a 3-day-old vine cutting. Note granular cytoplasm near head of nematode (×300). E) Gray-staining protoplasmic strands (arrows) in 2 giant cells converging to head of nematode (n)—fourth harvest (×300). F) Deep green-staining projections in giant-cell lumen beside nematode (n)—seventh harvest (×80). G) Massing of nuclei in giant cell: *p*, pyriform nucleus; *n*, elongate nucleolus—fourth harvest (×740). H) Mitotic nuclear divisions (arrows) in giant cell from root tip of 7-day-old vine cutting (×850). I) Collapsed giant cells in root (arrows)—third harvest (×60). J) Crack directly above group of giant cells near lateral root traces—fifth harvest (×40). K) Giant cell with contiguous hyperplastic parenchyma (P) toward the periderm (top of picture), centripetal abnormal xylem (ax), and lysis (l) of hyperplastic cells adjoining giant cell (gc)—fourth harvest (×120).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was used. Tissues were embedded in Fischer Tissuemat (melting point, 54°–56°C), and sections 12 μ thick were stained with safranin and fast green.

EXPERIMENTAL RESULTS.—*Nematode populations.*—Larvae of *M. incognita acrita* were most numerous in the soil samples. It was regarded as the principal nematode pathogen present. In addition, specimens of *Trichodorus christiei* Allen averaged 100 per pint of soil on August 20 and afterward. Previously, the numbers were much smaller. A few nematodes of the genera *Xiphinema, Helicotylenchus,* and *Tylenchorhynchus* also were present. The numbers varied from 1 soil sample to the next and the species were not determined. Fumigation reduced considerably the initial nematode population (Fig. 2, A). As the season progressed, populations increased in both treated and nontreated soil. There were significant differences in larval populations up through the third harvest but none thereafter. The populations reached a maximum of approximately 10,000 per pint of soil near the first of September and then slowly declined for the remainder of the season.

Influence of nematodes on plant growth.—Root-knot indices were consistently larger throughout the season on plants grown in nontreated than on those grown in treated soil (3.2 vs. 1.8, respectively). Little variation occurred in the relative indices through the season even though the nematode populations were similar during the latter part of the season.

Plant growth was greatly curtailed by nematode infection. Roots and tops of plants grown in treated soil were larger than those grown in nontreated soil (Fig. 2, B). In the fourth and subsequent harvests, the differences in root weights became increasingly greater as the season progressed. Also, the tops of plants from treated soil were larger than those of plants from nontreated soil; the differences became apparent in the second harvest, and top weights reached a maximum 2 weeks later than did those of plants grown in nontreated soil. The subsequent decline in weight of tops from treated soil was parallel with that of the controls.

Plants from treated soil had more enlarging roots with fewer cracks than did those from nontreated soil. The differences were fairly constant throughout the season. There were more cracks on roots from nontreated than on those from treated soil for harvests 5, 6, and 7 but not for harvest 4, in which cracking first appeared. At the last harvest, 15 and 50 per cent of the roots of marketable size from treated and nontreated soil, respectively, were cracked, and the average yield of U. S. No. 1 potatoes per plot was 18 lb. and 7 lb., respectively.

Correlations also were computed between root-knot indices and various growth responses. There were significant inverse correlations between root-knot index and each of the following: top weight, root weight, and number of enlarging roots. A significant positive correlation occurred between root-knot index and number of cracks and between the index and number of cracked roots per root system.

Histological studies.—1) *Infection courts.*—Primary root penetration by second-stage larvae occurred at the tips of young roots in the region of tissue differentiation. Larvae were observed to enter anywhere from the root cap back to the region of root-hair formation. Many nematodes were present among the cells close to the apical initials. Over 20 were found in 1 cross section of a root tip from a 5-day-old vine cutting (Fig. 3, A). Larvae moved primarily intercellularly through the cortex and intracellularly through the vascular tissue. Larvae apparently move rapidly through the cortex, as few were found in this tissue whereas they were numerous in the stele region. Evidence that larvae entered roots over a period of time was obtained by the observation that nematodes found in the vascular tissue 2 cm from the root tip were not always associated with giant-cell formation. Giant cells are initiated within a few hours after infection.

A second major nematode penetration occurred through the loose ruptured cells of enlarging roots where lateral roots emerged. Larvae that had been penetrating these lateral root ruptures were observed through the sixth harvest. In 1 piece of tissue, approximately 25 second-stage larvae were observed in the ruptured cortex and apical bud beside a lateral root trace (Fig. 4). From 10 to 40 nematodes in all

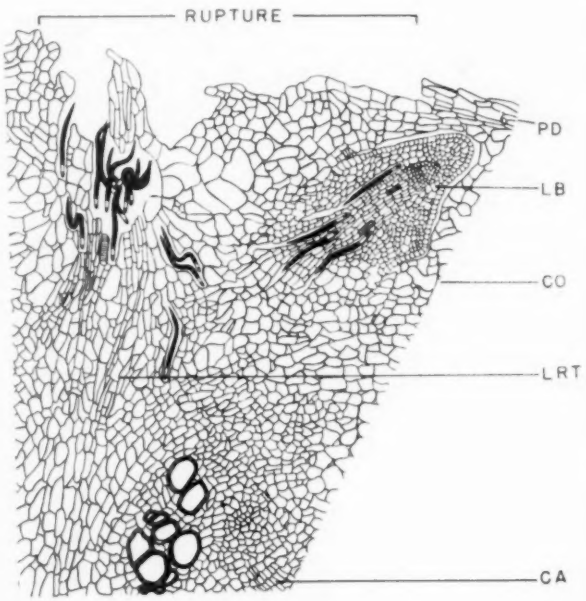

FIG. 4. Penetration of lateral root rupture by second-stage larvae. Composite camera lucida drawing (×50), from 3 serial sections, showing positions of nematodes as they pass through periderm (PD) rupture, along lateral root traces (LRT), through cortex (CO) into lateral bud (LB), and toward parenchyma in region of cambium (CA).

stages of development frequently were found in tissue surrounding lateral root traces in both cracked and noncracked roots (Fig. 3, B). The great majority of nematodes found in enlarging roots, with or without cracks, were associated with lateral roots. The nematodes were distributed from just beneath the periderm to deep in the vascular tissue and from among tissues of the lateral root to 2–6 mm longitudinally in the enlarging root.

A third major infection court was the broken surface of root cracks.[2] Infection resulting from this type of entry was obvious when cracks were large, and uncertain when cracks were small. Nematodes that entered crack surfaces were found singly rather than in groups. Single larvae penetrated the crack surface at random from just beneath the periderm to the very base of the crack deep in the root. Most of the nematodes remained within 10 cell diameters of the crack surface, but a few penetrated as much as 1 cm into the roots. Penetration through the crack surface was observed during the entire period that cracking was found but became more abundant as the season progressed. In the seventh harvest, over $\frac{2}{3}$ of the nematodes observed in roots with large cracks were not associated with lateral roots. In these roots, 62 nematodes were associated with lateral roots and 137 in crack surfaces in the 25-in. tissue sample examined.

Other minor penetration sites were the periderm of enlarging roots and the tissues of lateral roots. Nematodes found $\frac{1}{2}$ in. or more from lateral root traces or from crack surfaces were interpreted as having penetrated the periderm. Such infections were infrequent and usually consisted of single nematodes located anywhere in the root but mostly in the cambial zone. In many cases, these nematodes may have penetrated the root through lateral root ruptures and moved intercellularly to these scattered points. Occasionally, larvae appeared to have entered lateral roots and to have travelled through their vascular tissue into the enlarging root.

Late in the growing season, eggs laid deep in root tissues hatched and the larvae invaded surrounding potato tissue. At the last harvest, egg masses were surrounded with layers of cells that gave a positive suberin reaction to Sudan IV, a reaction typical of cork tissue (Fig. 3, C). Although a few free larvae were observed among the eggs, none had penetrated the cork layer. In these older roots, some third- and fourth-stage larvae were observed mixed with egg-laying females, an indication that secondary infection had occurred.

2) *Preferential feeding tissues.*—Giant-cell formation was associated with nematode feeding, and the tissues in which these cells formed indicated those tissues fed upon by the nematodes (Fig. 3, D). Giant cells were initiated in differentiating tissues of primary roots, lateral root and bud primordia, vascular and cortical parenchyma, secondary cambial zones, and tissues in lateral root traces.

The distribution and development of nematodes within the root varied with the ontogeny of the root. In root tips, the larvae came to rest in the stele parallel with the root axis and with the head oriented toward the stem. In enlarging roots with concurrent lateral root development, the nematodes came to rest in bud primordia, secondary cortex, cambial zones, and vascular tissues. Females located only a few cell diameters from the root or crack surface often extruded their egg masses to the surface. In mature roots, most nematodes were within 1 in. of the periderm. Most infecting nematodes in harvests 3 and 4 were enlarging larvae, whereas in harvests 5, 6, and 7 they were mainly mature females; however, second-stage larvae were observed in root tissues through the sixth harvest.

On the basis of giant-cell initiation, vascular parenchyma was a favorite feeding tissue for the larvae. In root tips, giant cells were formed before the vascular tissue had fully differentiated, but from their position they were initiated in xylem parenchyma adjacent to xylem vessels. First indications of giant-cell formation were found about 2 mm behind the root tip, although many second-stage larvae were closer than this to the apical initials. In small enlarging roots, giant cells were initiated at any point in xylem or phloem parenchyma, but most were in outer xylem tissue. Some enlarging roots from the first and second harvests contained as many as 60 maturing nematodes per linear inch, and functional vascular tissue was almost absent. Although the head regions of the nematodes were in the vascular parenchyma, the bulk of the swelled bodies rested in the secondary cortex. From the third harvest until the end of the season, the entire bodies of nematodes initiating infections in vascular tissue were embedded in vascular tissue. In older roots, giant cells were occasionally initiated in xylem parenchyma between existing groups of xylem vessels, and lateral expansion of giant cells was inhibited. Nematodes that penetrated crack surfaces often initiated giant cells in the vascular or storage parenchyma just beneath the damaged tissue, and egg masses frequently protruded from the crack surface.

Giant cells also formed in parenchyma cells in lateral root apices before the latter emerged from the primary root, in the cambial zones of enlarging roots, lateral root traces just inside the periderm, and in crack surfaces.

Infections in secondary cortex were frequently found throughout the studies. Giant cells initiated in the secondary cortex often extended into the cambial zone and vascular parenchyma as they enlarged. In several instances the giant cells were entirely in the secondary cortex.

3) *Formation of giant cells.*—First evidences of giant-cell formation in infected root tips were a granulation of protoplasm and a swelling of nuclei in parenchymatous cells around the head of larvae (Fig. 3,

[2] Hereafter, the area of broken cells exposed by cracking will be referred to as the crack surface.

D). Giant cells were initiated soon after infection. Root tips from 3-day-old vine cuttings had many small giant cells associated with larvae 2 mm from the root apex. In these roots, several giant cells (4 to 9) were frequently associated with a single larva.

The granulation of cytoplasm in affected cells was first noticed near the nematode's head. Upon enlarging, the giant cells filled with dense gray-staining cytoplasm that, at times, formed thick gray cytoplasmic strands in the lumina and converged at the nematode head (Fig. 3, E). As the nematodes matured, the cytoplasm stained light reddish and became more dense. After oviposition, the nematodes either died or stopped feeding as the giant cell protoplasm often degenerated and disappeared, especially in young enlarging roots, where the giant cells usually collapsed. Occasionally, no nematodes were associated with collapsed giant cells. It was assumed that males were responsible for this phenomenon, since males migrate from tissues at maturity whereas females are sedentary. Lack of a continual stimulus probably results in collapse of these cells where larvae developed into males. In old roots, the giant cells often did not lose their cytoplasm, but the cytoplasm remained very dense and stained reddish-brown, and the nuclei appeared shriveled (Fig. 3, F).

Giant cells were multinucleate, and the nuclei varied in size, shape, and other characteristics. Mitoses were observed in a young giant cell from a 7-day-old vine cutting (Fig. 3, H). The nuclei were larger than those in normal cells, sometimes approximately 100 times larger. Their shapes were spherical, elongate, pyriform, or dumbbell; they sometimes possessed projections. The very large nuclei contained a large amount of colorless nuclear sap with some dense-staining nuclear material. Giant cells contained around 50 to several hundred nuclei either scattered or in masses. The masses consisted of nuclei aggregated around a very dense, deeply red-staining, homogeneous core that apparently was nuclear material (Fig. 3, G). Some nuclei appeared to have coalesced with the central mass. Aggregated nuclei were often pyriform, with the neck projecting towards the center of the group. Many nuclei contained 2 or more nucleoli. In dumbbell-shaped nuclei there was a nucleolus in each arm. The nucleoli in massed nuclei converged towards the center, and some appeared free within the core. Some nucleoli were as large as normal nuclei and were variable in shape. In young giant cells, the nuclei possessed definite nuclear membranes, but in old infections they became shriveled and stained densely and the membrane was indistinct.

Walls of giant cells varied considerably during the season. Young giant cells in root tips from 3-day-old vine cuttings had a green-staining wall or membrane, except where the giant cells appeared to have been actively expanding. Their enlargement appeared to have resulted from the dissolution of adjacent parenchyma cell walls, from a fusion, or from multiplication of protoplasm and a subsequent deposition of wall around the enlarged lumen. As nematodes and enlarging roots matured, the walls became increasingly thicker, except in the areas where more parenchyma cells were being lysed. Walls of collapsed and empty giant cells were quite thick and stained deep red, almost black (Fig. 3, I); however, a few giant cells associated with dead or nonfeeding nematodes had almost no walls, suggesting disintegration.

Except for root tips and young roots, certain giant cells had green-staining, isotropic, nonpectin (negative to ruthenium red stain) knoblike structures projecting into the cell lumina (Fig. 3, F). These projections were numerous, were regularly spaced around the walls, and appeared granular on the projecting surface. In some giant cells, they were detached from the wall, were spherical in shape, and were scattered in the protoplasm. Walls, exclusive of the knobs, of these giant cells were anisotropic.

Giant cells assumed various forms, depending in part on the enveloping tissue. Those surrounded completely by xylem or cortical parenchyma were generally elongate, with the ends projecting longitudinally from the head of the nematode. Giant cells from adjacent infections intermingled or coalesced. In 1 instance, giant cells from 2 infections about 3 mm apart in xylem parenchyma were connected through a single narrow cell containing typical granular giant-cell protoplasm. Giant cells formed between xylem vessel groups were long and narrow, as lateral expansion was restricted. "Abnormal xylem," which is discussed next, also inhibited expansion and altered morphology of the giant cells. Giant cells formed in secondary cortex were usually smaller than those in cambial or vascular tissues. In small roots, xylem elements were often crushed or flattened by expanding giant cells.

4) *Other atypical tissues.*—A type of tissue designated "abnormal xylem" was associated with most infections (Fig. 3, K). Cells of this tissue were mostly devoid of contents, although a few appeared to contain nuclei. Abnormal xylem seemed to form directly from xylem parenchyma and was characterized by secondary wall thickenings of annular, reticulate, or pitted types. The cells assumed the shape of parenchyma cells but with no apparent intercellular spaces. Some cells bordering abnormal xylem had secondary thickenings on that wall adjoining the abnormal xylem. This differentiation response around giant cells occurred in the vascular tissue, cambial zone, and secondary cortex. It was most abundant around giant cells located in xylem parenchyma between vessel groups. In older infections, some groups of giant cells were nearly surrounded with abnormal xylem. Abnormal xylem, normal vessels, and sclereids were not absorbed by giant cells and restricted their expansion. A suggestion of abnormal xylem development was observed adjacent to freshly initiated giant cells in root tips from 3-day-old vine cuttings. In young enlarging roots, abnormal xylem was most concentrated around that part of the giant cell most distant from the head

of the nematode. The formation of these cells is apparently stimulated by nematode feeding, as they were not formed around a nematode body or egg mass. Occasionally, single abnormal xylem cells arose from any parenchyma cells adjacent to giant cells. Abnormal xylem was not always associated with nematode infection. In a few cases it was associated with necrotic lateral root traces in the absence of nematodes and appeared to be a wound response.

Nematode feeding also stimulated division of other parenchyma cells bordering giant cells. The parenchyma-type cells produced were very small, densely protoplasmic, and irregularly shaped; they formed a dense unoriented mass lacking intercellular spaces (Fig. 3, K). These cells were also concentrated close to the head of the nematode and were first noticed around giant cells in root tips of 7-day-old vine cuttings. Hyperplastic parenchyma was formed wherever giant cells were found and apparently was produced from any type of parenchyma cell. Sometimes the masses nearly surrounded giant cells; if abnormal xylem bordered giant cells on 1 side, hyperplastic parenchyma was on the other. None was found around collapsed giant cells. The largest masses of hyperplastic parenchyma appeared where giant cells seemed most active, especially in cambial zone and cortical infections. Several instances of these cells being incorporated into giant cells were observed.

Cork cells were associated with crack surfaces and nematode infections. Soon after a root cracked, cork formed, a few cell diameters beneath the exposed broken cells. It was first noticed around nematodes that penetrated root cracks in the fourth harvest; however, cork formation around nematodes was most abundant at the last harvest. Cork was found most readily around infections adjacent to cracks where active phellogen was present. Nematodes isolated by cork were mature, and contiguous host cells were necrotic. Similar nematodes located deep in the tissue were also walled off but only at the last harvest (Fig. 3, C). Actively growing larvae were usually not walled off, even at the last harvest. Loss of starch around nematode infections was similar to that near phellogen associated with cracks and other damaged tissue. This seemed to be a response to injury, since starch hydrolysis preceded the appearance of cork cells.

Enlargement of secondary cortical cells was associated with a few infections. Hypertrophy of cortical parenchyma cells occurred in root tips from 3-day-old vine cuttings when apical growth was inhibited by heavy nematode infection. In addition, the protoplasmic contents of apical cells were greatly reduced. In most enlarging roots, even concentrated infections caused little cell expansion; in fact, meristematic activity was probably reduced in the affected area as the roots became constricted. Most instances of enlarged cells were observed where nematodes had died or necrotic cells were present. Cortical-cell enlargement was generally associated with cambial zone or secondary cortical infections.

5) *Morphological changes in primary roots.*—Nematode infections in primary roots caused swelling of the entire root through a combination of hypertrophy and hyperplasia of cells (Fig. 1, A). As mentioned above, heavy larval infection inhibited apical growth and caused swelling of root tips. Single nematode infections caused small galls to form. As many as 60 enlarging nematodes per inch were observed in some primary roots, causing a general swelling in the infected region.

6) *Morphological changes in enlarging roots.*—Infection of enlarging roots caused either swelling, no visible effect, or root constriction. Occasionally, slight swelling of infected roots was visible in enlarging roots up to 1 cm in diameter, but as roots continued to enlarge, areas of heavy infection became constricted. Cracking was frequent after the fourth harvest and increased as the season progressed.

Infected and noninfected lateral roots, cell enlargement, hyperplastic parenchyma, giant cells, and the nematodes themselves all appeared at times to be associated with cracking. Lateral root ruptures were directly involved with 83 per cent of the large cracks and with 98 per cent of the small cracks. Since only a small fraction of large cracks was examined, a higher percentage than the above could have been associated with lateral roots. Nematodes were associated with 90 per cent of the large cracks, mostly in the crack surface, with 92 per cent of the small cracks, and with 63 per cent of the noncracked roots; there was little variation in the percentages with regard to time of harvest. More nematodes were present in roots with small cracks than in roots with either large cracks or no cracks. There were no differences between nematode populations in roots with large cracks and those in roots with no cracks. Giant cells, enlarged cells, and hyperplastic parenchyma were observed directly beneath fresh cracks (Fig. 3, J). In areas of heaviest concentration in small cracks, nematodes generally were clustered around lateral root traces near the center or widest part of a crack.

Discussion.—Although fumigation reduced the initial number of nematodes in the soil, there were no differences between treatments after the third harvest. Throughout the season, however, plant growth was significantly greater in treated than in nontreated soil. This indicates that heavy nematode infection in early season impairs plant growth for the entire season. Methyl bromide fumigation may have provided a better differential in nematode populations than did the treatment with vermiculite impregnated with D-D as used in this study; however, the results obtained demonstrate that heavy infestations of soil by *M. incognita acrita* reduce plant and root growth and increase root cracking of Porto Rico sweetpotatoes.

Many larvae entered the surface of large cracks. This possibly is related to the amount of fragile tissue exposed. Even so, larvae must be able to penetrate

this tissue through the cork, since the largest numbers were found in the last harvest. This is in contrast to the failure of larvae to penetrate cork around egg masses deep in the root.

Many nematodes were found in noncracked roots, and no nematode infections were found in some cracks. Factors other than nematodes also play a role in root cracking. It is possible that turgor pressure within the roots might be involved, as highly turgid roots have been observed to burst when jarred in harvest during cold weather; however, Ogle (13) found no differences in moisture content of cracked and noncracked roots. He further states that abundant nitrogen and high soil moisture increased cracking; these conditions produce high turgor and relatively thin-walled cells in plants. If nematodes inhibit localized cell division in actively growing roots, continued centripetal cell division and growth of underlying tissues may cause a rupture through the less active, infected cortical cells. Cracks were nearly always associated with infected lateral roots, and normal cell division and growth were undoubtedly impaired owing to the many larvae involved. In addition, necrotic host cells always accompanied such infections. Artschwager (1) states that lateral roots are arranged in straight vertical rows in enlarging roots. Many of the longitudinal cracks involved several lateral roots. El-Kattan and Stark (5) postulate that cracks form because vascular tissue enlarges more rapidly than do outer tissues following a period of adverse environmental conditions.

Abnormal xylem was associated with both giant cells and necrotic cells in traces of sloughed-off lateral roots. This suggests that these cells are formed as a response to injured xylem parenchyma rather than being peculiar to nematode infections; however, development was massive around giant cells, and none was present in crack surfaces. Giant cells seemed to influence abnormal xylem formation, especially near groups of xylem vessels. Abnormal xylem interfered only with expansion of old giant cells, since the nematodes were mature and laying eggs before abnormal xylem reached its full development.

In most suscepts, infection by root-knot nematodes usually stimulates swelling of roots, but in enlarging Porto Rico roots constriction is more often observed than swelling. Small swellings in primary roots caused by the growth and oviposition of the nematode are not visible in enlarging roots. In this variety, infection seems to inhibit rather than to stimulate hyperplasia in enlarging roots.

A large population of nematodes was never found in the center of large roots, even though small enlarging roots often contained enormous populations. Occasionally, single nematodes or a small group was observed in the center of mature roots. It is not uncommon, when assaying soils for root-knot nematodes 6–8 weeks after planting sweetpotatoes and other crops, to find fewer larvae than were present before planting. It is believed that this paucity of free larvae in the soil is due to their penetration of roots. Several more weeks elapse before free larvae are again found in the soil in large numbers. In the case of sweetpotatoes, potential storage roots may form and escape heavy infection during this period of low free nematode population whereas similar roots formed the first week or so after planting are heavily infected and fail to enlarge to marketable size.

The greenish-gray knoblike projections in giant cells are of interest. These were interpreted as a phase of giant-cell degeneration, although they were not observed in the degeneration of giant cells in young roots.

Several investigators have studied the histopathology of root-knot nematode galls in other plants, and many of the phenomena observed in this study have been described. Godfrey and Oliveira (7) reported that root-knot nematode larvae penetrated mainly the root tips of cowpea and pineapple, and that their heads came to rest in the endodermis or the outer periphery of the stele. Christie (2), working with root tips of tomato seedlings, made observations similar to those made in this study on larval penetration, movement of nematodes through root tissues, giant-cell enlargement, morphology of giant-cell nuclei, and formation of xylem from parenchyma cells surrounding giant cells. Christie referred to several papers that were not available for this review. In 1 reference (Tischler), mitotic nuclear divisions in young giant cells and nuclear division by amitosis or fragmentation in older ones were described. Linford (9) found that nematodes pierce giant cells during feeding and that substances are extruded from the stylet of the nematode. He further states that nematodes mature in certain plant roots without the formation of giant cells.

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HEAT ACTIVATION OF VIRUS INFECTIONS[1]

C. E. Yarwood

SUMMARY

When the leaves and stems of apple, bean, cowpea, cucumber, peach and sunflower were immersed in water at 45°, 50°, or 55°C for 2 to 500 seconds and weighed about 10 days later, the green weight of the plants was a good index of heat injury. The time of heating to produce 50 per cent reduction (ED_{50}) in the green weight of Pinto beans was about 300 seconds at 45° at dawn, 30 seconds at 50° at dawn, 72 seconds at 50° in the late afternoon, and 10 seconds at 55° at dawn. The growing points of plants were more sensitive to heat than were the primary leaves or cotyledons. Injury was greater when the plants were exposed to bright light immediately after heating than when they were placed in darkness and was greater for leaves oriented toward the sun after heating than for leaves oriented away from the sun.

When beans, cowpeas, or cucumbers were heated for about 20 seconds at 50° 6 hours to 3 days after inoculation with tobacco mosaic, tobacco ring spot, spotted wilt, peach yellow bud mosaic, or apple mosaic viruses, the appearance of necrotic lesions was usually delayed. On heated plants, lesions were larger and more numerous, and the number of systemic infections was greater than in unheated plants. With peach ring spot virus on bean, lesions were never found on unheated inoculated leaves, but adequately heated leaves showed well-defined lesions. Systemic infection of beans with spotted wilt virus has rarely been observed on unheated plants but was commonly induced by heat. Heating only the inoculated leaf, without heating the growing point or other parts of the plant, was usually sufficient to induce systemic infection.

It is suggested that ordinary inoculations with viruses produce more local infections than lesions and that the appearance of more lesions on heated leaves than on nonheated controls is the result of the activation of these otherwise latent infections. All cases of heat activation of infection may be associated with heat injury to the host.

Samuel (6) found that when plants of *Nicotiana glutinosa* L. were inoculated with tobacco mosaic virus and held at 35°C, systemic infection without clear local lesions resulted, whereas when similarly inoculated plants were held at 28°, local lesions without systemic infection resulted. Kassanis (3) discovered that heating plants at 36° for 2 days before inoculation increased the numbers of lesions formed on these plants. Yarwood (7) reported that when bean leaves were dipped in water at 45°C for 60 seconds and then inoculated with tobacco mosaic virus, the number of lesions resulting was about 7 times that which occurred on unheated plants.

In the present study, heat was applied to plants that were already infected, and the heating was continued for only a few seconds, during which time no significant multiplication or development of virus could have occurred according to our present knowledge. The term "activation" is used to denote this trigger-like action of the heat treatments. An abstract of preliminary results was published earlier (11).

METHODS.—Plants were grown in 4-in. pots of soil in a greenhouse where the temperature fluctuated from a minimum of about 19°C to a maximum of about 30° and averaged about 22°. At about 10 days after seeding, the plants were heated in trials of heat injury or were inoculated in trials of the effect of heat on infection. In the heating, single attached leaves or the entire tops of plants were immersed in a 3-liter water bath held at the desired temperature by means

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of an immersion heater. At the end of the heating period, the plants were returned to the greenhouse bench.

For trials of the effect of heat on injury to individual leaves, or on number, size, or time of appearance of local lesions, treated and control units were the opposite leaves of the same plant, and the value for the heated leaf was expressed as a percentage of that on the control leaf. For trials of the effect of treatment on injury to entire plants or on systemic infection, closely comparable plants were chosen before treatment, and the values for the treated plants were related to those of controls in the same trial. Because heat treatments had such profound effects on noninfected plants, and because heat injury could sometimes be confused with virus infection, it was desirable to have heated noninfected and heated infected plants in the same trial. In most cases, results of heating on virus infection were appraised on the basis of symptoms. With peach ring spot virus, however, the symptoms on peach and the early symptoms on bean were so indistinct in the early course of this work that it was necessary to assay these plants for ring spot virus by means of inoculations on cucumber, and even this test was not completely reliable, as some assay inoculations from infected plants failed. In some such trials no infection was detected in any plants, and such trials are omitted here.

Heat treatments sometimes resulted in an apparent increase and sometimes in an apparent decrease in infection. If these values are expressed as a percentage of the values for the unheated controls and averaged arithmetically, a distorted value results. On the basis of chance, the apparent effect of treatments would be randomly distributed around the 100 per cent level. For example, a 2-fold increase in infection would be as likely to result as a 2-fold decrease, and the average would be no net change. Yet if these values are converted to percentage (200 and 50 per cent, respectively) and averaged, the average value would be 125 per cent. To avoid this type of error, the percentage values were averaged geometrically on a logarithmic scale.

With tobacco mosaic virus on bean, the number of lesions per leaf on the untreated leaves averaged about 25; with the other viruses, the number of lesions per leaf was usually less.

Whereas the quantitative data on heat injury in this report are generally expressed as percentage reduction in green weight, a rating method would be more rapid. Also, a rating method was essential for following the time course of injury in the same leaf or plant. In 2 trials, a decimal rating scale of injury (0 = no injury; 10 = dead) was applied to plants immediately before weighing. The ED_{50} and the slope of the heat dosage response curve obtained by the rating method were almost identical to those obtained by the weighing method, and the rating method seems preferable for future studies.

RESULTS.—*Heat tolerance of healthy tissues.*—Heat injury was manifested as killing of tissues, changes in the orientation of primary leaves, stunting of new growth, and occasional mosaic-like patterns in the growth that occurred after treatment. On plants exposed to bright sunlight after heating, the upper epidermis was frequently killed whereas the lower epidermis appeared unchanged. Bean primary leaves that were heated during the day moved from the normal horizontal position down to an inclined or even vertical position, and sometimes the edges rolled upward.

Representative data on heat injury from 2 groups of tests are given in Figure 1, and summary data of most trials are given in Table 1. The heat tolerances of bean, cowpea, cucumber, and sunflower were about equal as indicated by this method. In all of these, the heat tolerance was less in the early morning than in the late afternoon. For example, the ED_{50} (heat dosage for 50 per cent reduction in green weight) was about 34 seconds at 50° for bean primary leaves at 5 a.m. to 9 a.m. and 72 seconds at 5 p.m. to 10 p.m. This diurnal difference in heat tolerance is presumed to be due to the diurnal difference in the carbohydrate content of the tissues, but Laude (5) did not consider this explanation adequate. The heat tolerance of plants as measured by the green weight of the new growth several days after treatment was less than that as measured by the green weight of primary leaves or cotyledons. Presumably the younger tissue of the growing point is more sensitive to heat than is the older tissue in the primary leaves.

The ED_{50} and the slope (change in injury per unit change of heat) of a dosage response curve should give all the essential information about it. The shape of these dosage response curves is not known with certainty, but is tentatively assumed to be a straight line on an arithmetic grid and is certainly close to a straight line over much of its length. The slope, expressed as change in percentage injury per second of exposure to a temperature of 50°, was 2.5 for primary leaves heated for short periods between 5 a.m. and 9 a.m. (Fig. 1), whereas it was about 1.3 for primary leaves at 5 p.m. to 10 p.m. These about represent the

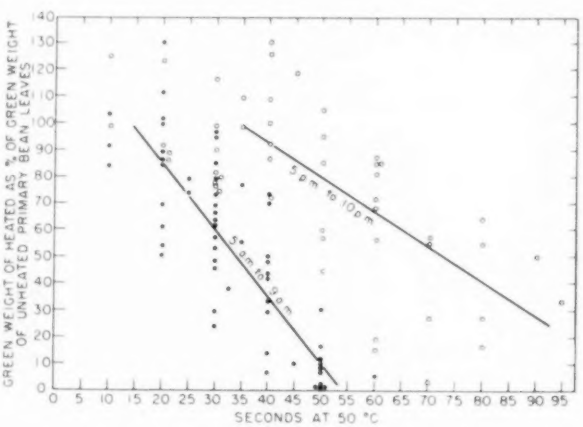


FIG. 1. Effect of duration and time of day of heating on injury to beans.

TABLE 1.—*Heat tolerance of plants subjected to hot-water treatments*

Host	Time at which plants were heated	Temperature (°C)	No. of trials	Seconds of heat treatment for 50 per cent reduction in green weight (= ED_{50})	
				Primary leaves or cotyledons	Growth beyond primary leaves
Bean	5 a.m. to 9 a.m.	45	4	430	205
		50	16	34	29
		55	6	6	5
	10 a.m. to 2 p.m.	50	6	44	44
	5 p.m. to 9 p.m.	50	9	72	40
		55	2	13	8
Cucumber	5 a.m. to 9 a.m.	50	5	41	17
	5 p.m. to 9 p.m.	50	7	57	26
	9 p.m.	55	1	15	14
Cowpea	4 a.m. to 8 a.m.	50	2		32
	5 p.m. to 9 p.m.	50	2		35
Sunflower	5 a.m. to 9 a.m.	45	2		450
		50	3		38
		55	2		9
	2 p.m. to 9 p.m.	50	6		60
		55	2		12
Apple	10 a.m.	50	1		110
Peach	7 a.m.	50	1		110
	4 p.m.	50	1		140

extremes of slopes encountered at 50°. At 45°C, the slopes were about 0.23; at 55°, they were about 12.

Heat injury was greatest when the leaves were heated in the early morning and then exposed to bright sunlight. Placing leaves in a dark chamber until the following morning reduced injury. For example, Pinto bean leaves heated for 50 seconds at 50° at 9 a.m. on April 5 and then exposed to sunlight showed an estimated 50 per cent injury at 3 days and 90 per cent injury at 5 days, whereas similarly heated leaves that were placed in darkness for 24 hours showed 5 per cent injury in 3 days and 10 per cent injury in 5 days. In another trial, the final green weight at 11 days after heating was 2.4 per cent of that of the controls for leaves left in sunlight continuously and 56 per cent of that of the controls for leaves held in darkness for 20 hours after heat treatment.

Orientation of the leaves with respect to the sun following heating was important in determining the extent of the injury. Because bean primary leaves turned down as a result of heating, usually to about 45° from the horizontal, the primary leaf on the south side of a heated plant received more sunlight than did the opposite leaf on the north side of the same plant. In 7 trials where heating was performed at 7 to 9 a.m., the heat injury of leaves placed in different positions after heating was followed by the decimal rating method. The average injury rating for leaves heated 30 to 45 seconds at 50° was 3.4 for leaves oriented to the north, 4.7 for leaves to the northeast, 4.9 for leaves to the east, 7.8 for the southeast, 6.0 for the south, 5.6 for the southwest, 2.7 for the west, and 3.9 for the northwest. The greatest injury for leaves oriented to the southeast probably is due to the fact that these leaves were most exposed to the sun immediately after heat treatment. The roots of beans and cucumbers growing in water cultures were much more tolerant to heat than were the tops, but the phenomenon was not studied intensively.

Number of lesions.—Heating of beans inoculated with tobacco mosaic virus, apple mosaic virus, alfalfa mosaic virus, peach yellow bud mosaic virus, or peach ring spot virus and heating of cucumbers inoculated with tobacco mosaic virus, spotted wilt virus, apple mosaic virus, or peach yellow bud mosaic virus at several hours after inoculation increased the numbers of virus lesions that subsequently appeared. Of the cases where some infections occurred without heating, the greatest increase due to heat was with apple mosaic virus on bean.

The combination most extensively studied was tobacco mosaic virus on Pinto bean. Properly timed exposures at temperatures from 31° to 55° increased the numbers of lesions that subsequently developed. Increases due to heating at 31° are less readily acceptable as activation as here defined, since 31° is about the optimum for virus increase (4); leaves must be held at 31° in humid air for several hours (several generation times) in order for an increase in numbers of lesions to be clear. Exposure of leaves to 37° for 9 hours in humid air at 1 hour after inoculation increased lesion development, but holding leaves at 37° for 48 hours prevented subsequent manifestation of lesions, though the leaves were not severely injured. Holding inoculated leaves in humid air at 42° for 80 minutes beginning 1 hour after inoculation increased the number of lesions that eventually developed an average of 6-fold in 2 trials. For 45° water treatments, the optimum exposure time appeared to be

about 120 seconds at 10 hours after inoculation, but this was not clearly established. Even in 530 paired treatments at 50°, the results were extremely erratic between replicates. Summary results of all 50° treatments are given in Figure 2. Heating at 50° immediately after inoculation decreased lesion formation by about 40 per cent for a 5-second treatment, 88 per cent for a 20-second treatment, and 98 per cent for a 40-second treatment. At 2 hours after inoculation (1 trial), 10 seconds at 50° reduced lesion formation by 30 per cent, 20 seconds reduced it by 50 per cent, and 40 seconds reduced it by 80 per cent. Heating at 2.5 to 5 hours after inoculation (7 trials) had no apparent effect up to 10 seconds, but 40 seconds reduced lesion formation by about 50 per cent. Heating at 50° at 5 to 10 hours after inoculation was optimum for the formation of lesions caused by tobacco mosaic virus, and 45 seconds at 50° gave about 3 times as many lesions as appeared on unheated leaves. Treatments for 10 to 20 seconds at 10 to 24 hours after inoculation increased lesion formation by about 50 per cent, and treatments at 24 to 72 hours gave only doubtful increases.

At 14 hours after inoculation in 1 trial, heating at 55° for 3 seconds gave a 36 per cent increase in the number of lesions that subsequently developed, heating for 6 seconds gave a 17 per cent increase, heating for 9 seconds gave a 17 per cent increase, and heating for 12 seconds (severe injury) gave a 69 per cent decrease. The optimum duration of heating at the optimum age of infection was not adequately determined but appeared to be about 150 seconds at 45°, 35 seconds at 50°, and 4 seconds at 55°.

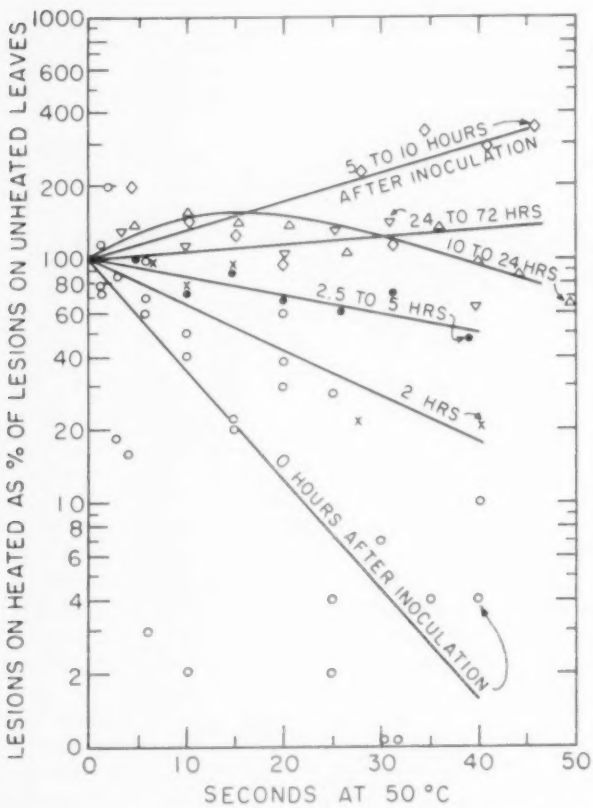


FIG. 2.—Relation of age of infection and duration of heat treatment to number of lesions formed by tobacco mosaic virus on Pinto bean. Only the data points for 5 trials (28 pairs) of heating immediately after inoculation are given. The other curves are averages of a total of 502 paired comparisons.

Time of day of heating, time of day of inoculation, or time of year of trial had no clear effect on the response of inoculated leaves to heat treatments. The increase from heating appeared to be greater on old than on young leaves, but this was erratic. In 2 trials, the number of lesions on leaves heated for 30 seconds at 50° at 10 hours after inoculation exceeded that on nonheated leaves by an average of 10 per cent for 6-day plants, 250 per cent for 10-day plants, and 330 per cent for 16-day plants; however, 16-day plants usually yielded too few lesions for satisfactory experimentation.

Inoculations of bean with apple mosaic virus without phosphate were usually unsuccessful (8). Heating leaves at several hours after inoculation resulted in lesions in many cases where no lesions resulted on the unheated controls, and differences as great as 72 lesions on a heated leaf and 1 lesion on the control leaf have been obtained when leaves were inoculated without phosphate. Results without phosphate were so erratic, however, that 0.05 to 0.5 per cent K_2HPO_4 was usually added to inoculum to give reliable infection, even though the response to heat on leaves that were inoculated with phosphate was less than for leaves inoculated without phosphate. The effect of heating bean leaves that had been inoculated with apple mosaic virus was studied in 60 trials in about half of which low or no infection resulted. In trials where lesions averaged 5 or more per leaf on nonheated leaves, Pencil Pod or Bountiful bean leaves heated for 10 to 150 seconds at 45° or for 2 to 40 seconds at 50° at 8 to 24 hours after inoculation averaged 3.8 times that number of lesions in 167 paired comparisons. The observed average difference was greatest (14-fold) for treatments of 100 seconds at 45°. For treatments at 50°, the greatest difference resulted from about 8 seconds of exposure, and treatments at 10 to 16 hours after inoculation resulted in greater differences than did treatments at shorter or longer intervals following inoculation.

Size of lesions.—Lesions of tobacco mosaic virus on Pinto bean under greenhouse conditions usually ceased growth when the lesions reached about 300μ in diameter. Larger lesions developed on heated leaves than on nonheated ones, and the effect of heat treatments on lesion size was more consistent than was the effect on numbers of lesions; large lesions frequently resulted from heat treatments that resulted in fewer lesions than developed on nonheated leaves. Postinoculation treatments were more successful in producing lesions larger than normal than were preinoculation heat treatments (10), but the opposite

applied for numbers of lesions. In all 74 pairs of comparisons of the effect of postinoculation heating on lesion size with tobacco mosaic virus on bean, the average diameter of the lesions was 114 per cent of the controls for 10 seconds at 50°, 125 per cent for 20 seconds, 160 per cent for 30 seconds, and 180 per cent for 40 seconds. The maximum difference in a single paired comparison was 350 per cent (control lesions averaged 330μ, and lesions on the opposite leaf heated 30 seconds at 50° at 11 a.m. on January 30 at 24 hours after inoculation averaged 1500μ). The optimum time of heating was at about 20 hours after inoculation. The average lesion size on heated leaves was 115 per cent of that for the controls on leaves heated at 5 hours after inoculation, 150 per cent for leaves heated at 7.5 hours, 200 per cent at 20 hours, 160 per cent at 40 hours, and 140 per cent at 80 hours. Although numbers and size of lesions were affected somewhat similarly by heat and are therefore correlated in a gross way, there was not a good correlation between numbers and size of lesions in individual paired treatments.

Postinoculation heating of inoculated leaves also resulted in unusually large lesions with apple mosaic virus on Bountiful and Pencil Pod bean and with tobacco mosaic virus, spotted wilt virus, or peach yellow bud mosaic virus on cucumber cotyledons.

Virus assay.—The effect of heat treatments on local virus development was also revealed by virus assay. Disks of control and heated tissue from opposite leaves, of which one was a control and the opposite one heated, were ground in water and used as inoculum. Even when heated leaves had no more lesions than did the nonheated controls, the heated leaves contained much more virus than did the controls. In 16 paired comparisons, the number of assay lesions of tobacco mosaic virus from the heated leaves ranged from 0.9 times as many as from the control leaf for a leaf heated 1 second at 50° at 26 hours after inoculation to 23 times as much for a leaf heated 40 seconds at 50°; they averaged 4.8 times as much as the controls in all trials.

Delay in necrosis.—Necrotic virus lesions were usually slower to appear in heated than in nonheated tissues. This effect was greater with tobacco mosaic virus on bean and with peach yellow bud mosaic virus on cowpea than with other virus-host combinations studied. In 1 set of inoculations of bean with tobacco mosaic virus that resulted in 587 lesions, none of the lesions eventually formed had appeared on the nonheated leaves in 36 hours, 42 per cent had appeared in 47 hours, 55 per cent in 54 hours, 69 per cent in 70 hours, 77 per cent in 78 hours, 86 per cent in 94 hours, 93 per cent in 118 hours, and 100 per cent in 168 hours. On the same plants but on the opposite leaves, which were heated for 35 seconds at 50°C at 13 hours after inoculation, no lesions had appeared in 36 hours, 2 per cent of the lesions had appeared in 47 hours, 8 per cent in 54 hours, 49 per cent in 70 hours, 65 per cent in 78 hours, 79 per cent in 94 hours, 86 per cent in 118 hours, 98 per cent in 168 hours, and 100 per cent in 192 hours. In this trial, the time required for the appearance of 50 per cent of the lesions was about 51 hours for control leaves and 71 hours for heated leaves.

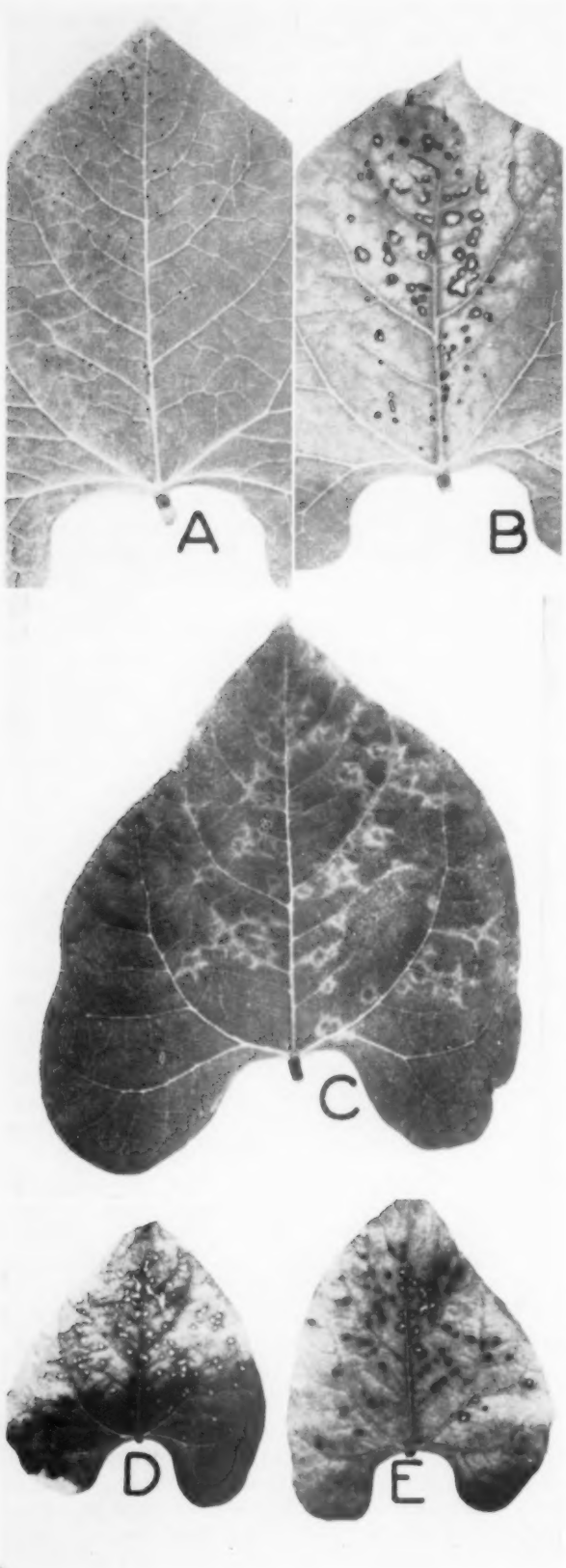
With peach yellow bud mosaic virus in cowpea, local lesions appeared on the inoculated leaves in about 4 days, systemic shock symptoms in the new growth in about 7 days, and necrosis of the main stems in about 8 days. Appearance of lesions and of systemic shock was delayed by heat, but the effect of heat on stem necrosis was most striking. Plants treated 20 to 50 seconds at 50° at 3 days after inoculation frequently showed no stem necrosis at 8 or 10 days whereas nonheated plants showed severe necrosis. Even at 18 days after inoculation and 15 days after heating, stem necrosis was sometimes much less on heated than on control plants.

Manifestation of latent lesions.—Inoculations with a strain of peach ring spot virus from an apricot tree grown in Solano County, California, produced systemic infection in several varieties of bean. Systemic symptoms were sometimes manifest as necrosis of petioles, stems, and veins; as slight mosaic in new growth; as slight twisting of main stem; and as a very light set of flowers and fruit, but symptoms did not usually appear before 10 days after inoculation and were erratic in appearance. Local lesions on inoculated leaves were never observed on nonheated leaves. When inoculated leaves were heated for 30 seconds at 50° (long enough to give slight injury) at 2 to 11 days after inoculation, virus lesions were sometimes clearly apparent 2 days later as green islands or as chlorotic halos (Fig. 3, C). These zones were about 0.7 mm in diameter for leaves heated at 2 days after inoculation, 3 mm for leaves heated at 6 days after inoculation, and 18 mm for leaves heated at 11 days after inoculation in different tests. This heat effect was most pronounced on the variety Oregon Giant, it was less so on Pencil Pod, and no clear lesions were found on Pinto, though each of these varieties became systemically infected. With another strain of peach ring spot virus (Fig. 3, D, E), the heat effect was well expressed on Pencil Pod.

With peach yellow bud mosaic virus on bean, infection areas normally appeared on the inoculated primary leaves in about 7 days as chlorotic or necrotic lesions about 4 mm in diameter. They appeared much sooner than this on heated leaves.

The above 2 examples are exceptions to the generalization made earlier that heating delays the appearance of symptoms.

This manifestation of latent lesions as a result of heat treatment is believed to be similar to the manifestation of latent lesions by staining with iodine (2, 6), in that both probably depend on the accumulation of carbohydrates in the lesion area. With the heat-induced lesions, as studied here, the detection treatment is much easier and quicker than the iodine treat-



ment, and the leaves are not sacrificed as with the iodine treatment.

Systemic infections.—Heating of inoculated plants for 10–40 seconds at 50° or for 30–180 seconds at 45° has resulted in systemic infections of beans and cucumbers with viruses that would otherwise remain localized in these plants, or it has increased the percentage of plants that became systemically infected. Tobacco mosaic virus normally produced only local lesions on cucumber, but systemic symptoms resulted in 2 of 90 heated plants; virus was recovered from one of these plants. This result could not be repeated.

Tobacco ring spot virus commonly gave local lesions and systemic infection in young beans but only local lesions on beans more than about 12 days after seeding. Under conditions where systemic infection did not normally result in nonheated plants, a high percentage of systemic infection resulted in heated plants. In 14 such trials, 14 per cent of the nonheated control plants became systemically infected, as did 55 per cent of the plants heated 10 seconds at 50°, 86 per cent of the plants heated 20 seconds, and 94 per cent of the plants heated 50 seconds. Systemic infection resulted in plants heated at 1, 2, 3, or 4 days after inoculation, with no well-defined optimum.

Systemic infection by spotted wilt virus on Pinto bean has been seen only once in several hundred nonheated plants, but the heating of plants for 30 seconds at 50° at 2 days after inoculation commonly caused systemic infection. Commonly only a portion of the new growth showed symptoms (clearing of veins; down curling of leaves; necrosis of stems, petioles, and veins; chlorotic or necrotic spotting), and the virus could be isolated only from plant parts showing symptoms.

Inoculations with spotted wilt virus on very young cowpea plants commonly produced local lesions and systemic infections, but similar inoculations on older plants usually resulted in local lesions only. Heating such older plants resulted in a high level of systemic infection. For example, in 2 representative trials, inoculations on plants 10–17 days after seeding resulted in systemic infection in 2 of 22 nonheated plants, but heating similar plants for 10–60 seconds at 50° at 1 to 5 days after inoculation resulted in systemic infec-

FIG. 3. Heat activation of virus infections. A, B) Twin primary leaves of Pinto bean inoculated with tobacco mosaic virus 10 a.m. January 29 and photographed February 8, 1957. A) Control. B) Heated 30 seconds at 50°C at 10 a.m. January 30 at 24 hours after inoculation. C) Primary leaves of Oregon Giant bean inoculated with a strain of peach ring spot virus 5 p.m. May 10, heated 30 seconds at 50°C at 8 p.m. May 12, and photographed May 17. The unheated control leaf (not illustrated) showed no lesions. D, E) Primary leaves of Pencil Pod beans inoculated 5 p.m. June 15 with a different strain of peach ring spot virus from leaf C, photographed June 21. D) Heated 50 seconds at 50°C at 6:30 a.m. June 17. E) Heated 9 seconds at 55°C at 6:30 a.m. June 17. The unheated control leaves (not illustrated) showed no lesions.

tion in 29 of 36 plants. Heat caused the formation of large lesions by spotted wilt virus on cucumber cotyledons, but no effect on systemic infections due to heat was noted.

In 1 trial with peach yellow bud mosaic virus in Dwarf Horticultural bean, the number of systemic infections in 10 control and 10 heated plants was 0 and 4, respectively, at 9 days after inoculation, 1 and 8 at 13 days after inoculation, and 7 and 10 at 19 days after inoculation. The optimum time for systemic infection with this virus-host combination was about 160 seconds at 45° at 2 days after inoculation. In 1 trial with Himalaya blackberry mosaic virus (1), believed to be closely related to peach yellow bud mosaic virus, 3 of 16 control Pinto beans became systemically infected, and 14 of 16 heated plants became systemically infected.

With peach ring spot virus in Pinto and Pencil Pod beans, systemic infection commonly resulted without heating, but a higher percentage of systemic infections resulted in heated than in nonheated plants, and the virus was recovered sooner from the new growth of heated plants than from the new growth of nonheated plants. In 2 trials with alfalfa mosaic virus in cucumber, 1 of 10 control plants showed systemic symptoms, whereas 9 of 10 heated plants showed systemic symptoms. Heat also increased systemic infection by alfalfa mosaic virus in Pinto bean and by apple mosaic virus in cucumber.

Repeated heat treatments.—Since single heat treatments produced such profound effects, it was thought that heat treatments every 12, 24, or 48 hours might cause still greater effects on numbers of lesions, size of lesions, delay in necrosis, or systemic infections. There was some delay in necrosis as a result of successive heatings, but in all cases the first heating was the most effective, and no type of repeated heating tested prevented necrosis with tobacco mosaic virus on bean or with peach yellow bud mosaic virus on cowpea. Repeated heat treatments did not seem to affect appreciably the number of lesions or number of systemic infections.

Localization of the heating effect.—Some Pinto beans inoculated with tobacco ring spot virus and some inoculated with spotted wilt virus were heated only on the inoculated leaves, some only on the noninoculated primary leaves opposite the inoculated leaves, and some only on the new growth; with some, the entire above-ground parts of the plants were heated. Systemic infection occurred about as frequently when only the inoculated leaves were heated as when the entire plant was heated, and heating the noninoculated primary leaves or the growing points did not increase infection. In such systemic infections, therefore, the effect of heating can be better explained as increasing the invasiveness of the virus than in increasing the susceptibility of distant tissues.

Dry heat.—With hot-water treatments, the actual time the leaf reaches the desired temperature can be more precisely known, and the experimental manipulations more readily carried out, than with dry heat. Because of the known deleterious action of water on virus infection (9), however, it was felt essential to make trials with dry heat. The method used was to fit waterproof plastic envelopes over the leaves and lower the leaves in the plastic envelopes into the water bath. These trials showed that 50° dry heat for 40 seconds immediately after inoculation prevented formation of tobacco mosaic virus lesions on bean, and 50° dry heat for 30 seconds at 5 hours after inoculation resulted in more lesions than appeared on the controls, much as with the hot-water treatments. Therefore, for heat treatments at 5 or more hours after inoculation, the results of these trials are considered as predominantly due to heat and as not seriously complicated by the water effect.

Discussion.—The mechanism of heat activation of virus infections is not understood, but it is appropriate to speculate. The appearance of more lesions on bean leaves heated at 24 hours after inoculation with apple mosaic virus than on comparable nonheated leaves is presumed to result from the increase in invasiveness of the virus and/or in the necrosis caused by many infections that would not otherwise be apparent. Apple mosaic virus has a half life of about 5 minutes *in vitro* (8) under greenhouse conditions, yet leaves heated at 5–24 hours after inoculation have developed 10 times or more lesions than developed on the controls on several occasions. It is highly improbable that the naked virus had remained latent for 24 hours, especially since inoculations at more than 1 hour after abrasion have been unsuccessful. Therefore, it is suggested that in ordinary inoculations many more local infections occur than is indicated by the number of lesions counted. Perhaps better means than the hot-water method might be devised for activating these infections, especially since water is deleterious to the infection process.

The most promising practical uses of the method appear to be in inducing local lesions in infections where lesions normally do not appear, as in the case of peach ring spot virus on bean, and in inducing systemic infections in cases where only local lesions normally occur, as with spotted wilt virus on bean.

Part, if not most, of the effects noted here are probably related to the increased susceptibility of plants to several viruses and fungi as a result of heating the leaves before inoculation (3, 4, 10). With preinoculation heating, the responses of fungi and viruses were superficially similar. With postinoculation heating, the response of fungi and viruses appeared basically different. Heat dosages that normally activated virus infections (e.g. 120 seconds at 45°, 30 seconds at 50°) consistently killed *Uromyces phaseoli* (Pers.) Wint., *Erysiphe polygoni* DC., and *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav. in infected tissue.

Virus in plants can also be inactivated after infection (4), but this was not encountered with certainty in the

present study. In the several cases where virus lesions did not appear following heat treatment of inoculated plants, this failure of the plants to manifest symptoms was associated with severe injury to the host, and the lack of symptoms could have been due to lack of susceptible host tissue rather than to inactivation of the virus.

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DISSEMINATION BY ATMOSPHERIC TURBULENCE: SPORES OF PERONOSPORA TABACINA [1]

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SUMMARY

The number of spores of *Peronospora tabacina* per cubic meter of air 46 cm above the ground was measured hourly during 2 blue mold outbreaks in tobacco seed beds. In the first, spores were trapped above a 46-m^2 area containing more than 10^4 lesions. Following a night with drizzle, the clouds broke and the air became drier as the sun rose between 4 and 5 a.m.; air-borne spores first appeared in quantity at this time and reached a maximum concentration within the hour. The day was sunny and gusty, with no air-borne spores detected at or after 8:30 a.m. On cloudy days, spores first appeared in quantity at 6:30 a.m. and reached maxima of 432–5000/m^3 at 8:30–10:30 a.m.; very few appeared at or after 2–7 p.m. In the second outbreak, spores were trapped above a 6.7-m^2 area containing about 200 lesions. On a clear day, spores appeared in quantity and reached a maximum of 90/m^3 between 5:30 and 7:30 a.m.; none appeared at or after 10 a.m. On 2 clear days following this, maxima of 6–12/m^3 appeared between 1 and 5 a.m. Evidently the maximum number of spores are dispersed early in the morning, and few or no spores are air borne during the afternoon or night. If the spores are assumed to be dispersed according to the statistical theory of turbulence, with variances in the horizontal directions of $\frac{X^{7/4}}{2}$ and in the vertical direction of $\frac{2X^{7/4}}{9}$, then the lesions must disseminate a maximum of about 10^5 spores/day (X is the distance downwind).

An understanding of the influence of weather upon the spread of fungi pathogenic to crop plants is one of the important problems in agricultural meteorology. The number of spores disseminated per lesion in the field and the hour of dissemination are critical. These facts are fundamental in the appraisal of risk of disease loss, in the forecast of multiplication of pathogens, and in the design of disease-control measures. The hourly concentration of air-borne spores of *Peronospora tabacina* Adam has been measured above known numbers of tobacco blue mold lesions and is reported here.

A source of Peronospora spores can be characterized from the distribution of primary infection (7); however, the estimate of the number of spores disseminated depends upon the hypothesis of turbulent transfer one assumes. With any given hypothesis, further difficulties arise because the number of spores disseminated cannot be estimated independently from the infectivity of the spores and the size of the plant

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part infected (6). A final uncertainty is caused by the dependence of the estimate of source strength upon the choice of a value for the proportion of spores trapped by foliage; although this proportion can be estimated independently, it cannot be estimated precisely (2). Actual measurement of the quantities of air-borne spores produced by a lesion is the first step in unravelling this complex.

The hour of dispersal can be predicted from de Bary's (1, p. 72) observations of *Phytophthora infestans* (Mont.) d By.: "Where filiform sporophores rise free into the air, a further mechanical arrangement is found which greatly assists the shedding and scattering of the abscised spores. . . . (The sporophores) are so highly hygroscopic that the slightest change in the humidity of the surrounding air, such for instance as may be caused by the breath of the observer, at once produces changes in their turgescence and torsion; the latter give a twirling motion to the extremity of the gonidiophore and the ripe spores are thereby thrown off in every direction." De Bary's observations were either overlooked or ignored by most workers until Pinckard (4) called attention to them 5 decades later. Pinckard found that if infected tobacco leaves were kept in a saturated atmosphere, sporulation of *Peronospora tabacina* occurred, but the sporangia were not released. Then, when drying began, the crown of each fruiting structure and the sterigmata-like structures bearing the spores began to twist and turn. If moist air appeared, the motion was reversed. The net effect was the release of mature, not immature, spores. These observations led Pinckard to suggest that the pathogen was disseminated following, not during, a continuous rain or atmospheric saturation.

Hourly measurements of the concentration of air-borne spores were made above lesions following blue mold outbreaks of known extent in 1955 and 1956. These permitted us to estimate dissemination per lesion and its daily cycle.

Materials and methods.—Spore concentrations in the air 46 cm above the soil were determined with an automatic volumetric spore trap (3). A centrifugal fan driven by a motor powered by 30 volts from storage batteries provided a reliable and portable means of drawing air into the trap. The spores of *P. tabacina* were identified and counted microscopically; the counts were converted to concentrations in the air by means of anemometer observations and of published efficiencies of the trap (3).

We had the good fortune of studying 2 strikingly different epiphytotics. In 1955, the trap was placed in seed beds of tobacco severely infected with *P. tabacina;* the beds were provided by Dr. P. J. Anderson. Three parallel seed beds, 18 × 2.4 m, were arranged 1.2 m apart and with their long axes north to south. About $\frac{1}{3}$ of the plants in the west bed had lesions on May 31. In the other 2 beds, leaves without lesions could rarely be found; at least 70,000 lesions must have been present in each of these beds. The wet burlap cover was removed from the beds at 1 p.m. on June 1 and the trap placed in operation at 3 p.m. at the center of the east bed. Successful observations were made on 1 sunny and 2 cloudy days.

In 1956, the outbreak of blue mold was much less severe than in 1955. The trap was placed in the center of a 1.8 × 3.7-m area of plants in a seed bed. On June 15, about 100 young lesions were evident. The trap was operated during that and following nights and early mornings; the seed bed was covered with a wet white canvas during the day. By June 20, the number of lesions had increased to about 200; subsequently, the trap was operated continuously, with the beds uncovered. Most of the lesions were in the center 4 m^2 of the bed.

Results.—The brief, but significant, series of observations in 1955 is reproduced in Table 1. These data are employed in the discussion for the estimation of the daily cycle of dissemination.

The series of observations in 1956 is most suitable for the estimation of production per lesion. Few spores were found in the air. During the first 5 days of study, spores were observed only twice in a total of 76 observations, and then in concentrations of less than 10/m^3. These 5 days were without measurable rain and had maximum temperatures of 70°–96°F. On the sixth day, rain fell for about 20 hours. The seventh day dawned hot and dry. Spores were observed in the air by 5 a.m.; they reached a maximum concentration of 90/m^3 at 6 a.m.; and none appeared at or after 10 a.m. The following morning, the eighth, as many as 6 spores/m^3 were air borne between 1 and 2 a.m., a time of fluctuating humidity. After another warm dry day, a few spores were disseminated near midnight and some more (4–12 m^3) between 4 and 6 a.m. the next morning. The maximum temperatures were all above 75° on these days. Although the observations were continued until the sixteenth day, only a few spores were seen; most of these appeared at 5 a.m. on the thirteenth and fourteenth days following 2 showers. Thus, the small epiphytotic of 1956 was in sharp contrast to the severe one of 1955.

Discussion.—The number of spores disseminated per lesion in the field can be deduced from the observations in 1956. More ample data are now at hand than when an earlier estimate was made (6).

To obtain the number of spores disseminated per lesion, we must perform 2 operations. First, the spore-trap data must be converted to spores per hour per cm^2 of vertical plane. Second, the observation at a height of $z = 40$ cm above the lesions must be converted to an estimate at foliage height, $z = 0$. The lesions at 6 cm above the soil have been taken as the reference $z = 0$.

The first conversion can be accomplished easily by dividing the number of spores trapped per hour by the efficiency of the trap and the area of the orifice through which the spores were drawn. The maximum spore dissemination in 1956, at 6 a.m. on the seventh day, was 145 spores/cm^2/hour. The total dissemina-

tion per day was generally 3 times the maximum rate (see Table 1); thus, about 500 spores must have passed through the vertical 1-cm² area 40 cm above the foliage in 1 day.

The conversion from 40-cm height to plant level requires assumptions concerning the distribution of the lesions in the plot and the manner in which turbulence transports the spores. Let us assume that the lesions were evenly distributed over the 4-m² area ABCD (Fig. 1). If the wind blew along the x axis, most of the spores that reached the trap 40 cm above point T came from the area TBC. The statistical theory of turbulence has been useful in analyzing dissemination (2, 5). According to this theory, the num-

TABLE 1.—*Time, weather, and air-borne spores of Peronospora tabacina in 1955*[a]

Date	Hour	Sky cover	Weather	Temperature (°F)	Wind (Miles per hour)	Spores/m³
6/1	3:30 p.m.	B		67	17.3	0
	4:30	V	Shower	64	19.6	0
	5:30-8:30	V-B		63-60	18.4-10.4	0
	9:30-10:30	B		58	9.2-11.5	2
	11:30	B		56	8.1	0
6/2	12:30-3:30 a.m.	B		54-52	5.8-8.1	0
	4:30	B		54	12.7	4
	5:30	B		54	11.5	0
	6:30	B		55	9.2	80
	7:30	B		57	10.4	1140
	8:30	B		58	11.5	5000
	9:30	B		62	17.3	3600
	10:30	V		61	17.3	1320
	11:30	V		61	15.0	820
	12:30 p.m.	V		63	18.4	800
	1:30	V		64	19.6	250
	2:30	B		64	17.3	92
	3:30	V		64	11.5	88
	4:30	V		63	15.0	16
	5:30	V		63	15.0	4
	6:30	V		62	13.8	16
	7:30-9:30	V-B		60-58	10.4-16.1	0
	10:30	V		58	11.5	4
	11:30	V		58	11.5	4
6/3	12:30-1:30 a.m.	V	Drizzle	56-55	10.4-11.5	0
	2:30	V		55	11.5	8
	3:30	V		55	12.7	0
	4:30	B		56	15.0	240
	5:30	S		55	13.8	208
	6:30	S		57	17.3	132
	7:30	S		58	19.6 G	16
	8:30 a.m.-3:30 p.m.	S-B		62-72	20.7-26.5 G	0
	4:30-11:30	S-B		72-52	15.0-0	0
6/4		V		45-70	4.6-10.4	M
6/5		S-B	Showers (8:00-12:00 p.m.)	58-75	3.5-13.8	M
6/6	12:30-11:30 a.m.	S-B		61-77	M	M
	12:30-2:30 p.m.	B		79-82	13.8-15.0	0
	2:30-11:30	S-B		86-61	3.5-17.3	M
6/7	12:30 a.m.-12:30 p.m.	V		58-62	4.6-10.4	M
	1:30 p.m.	V		62	8.1	8
	2:30-5:30	V		62-60	10.4-13.8	0
	6:30	V		59	5.8	4
	7:30-9:30	V-S		58-56	4.6	0
	10:30-11:30	V	Fog	53-54	10.4-4.6	0
6/8	12:30-5:30 a.m.	V	Fog	52-54	3.5-5.8	0
	6:30	B	Fog	55	6.9	28
	7:30	B	Haze	57	15.0	56
	8:30	V		58	15.0	132
	9:30	V		58	17.3	364
	10:30	V		58	17.3	432
	11:30	V		57	18.4	116
	12:30 p.m.	V		56	13.8	12
	1:30	V		56	17.3	0

[a] V, overcast sky; B, broken clouds; S, scattered clouds; G, wind in gusts; and M, missing data. Weather observations taken by U. S. Weather Bureau at airport 4 miles away.

ber of spores (s) reaching the trap at any instant from the 1 cm² at point P, would be

$$s = \frac{3q}{2\sigma^3(2\pi)^{3/2}} e^{-\frac{1}{2}\left(\frac{x^2}{\sigma^2} + \frac{y^2}{\sigma^2} + \frac{9}{4}\frac{z^2}{\sigma^2}\right)}$$

where x, y, and z are the distances from the center of a puff of q spores disseminated at P. The variance in the vertical is set at 4/9 of the variance σ^2 in the other directions (5). Figure 2 is a diagrammatic representation of the puff or cloud from P at an early moment as it drifts downwind and later as it passes the trap at T.

S, the number of spores passing through 1 cm² at trap height over an extended time (1 morning) and from all of area BTC is calculated as follows:

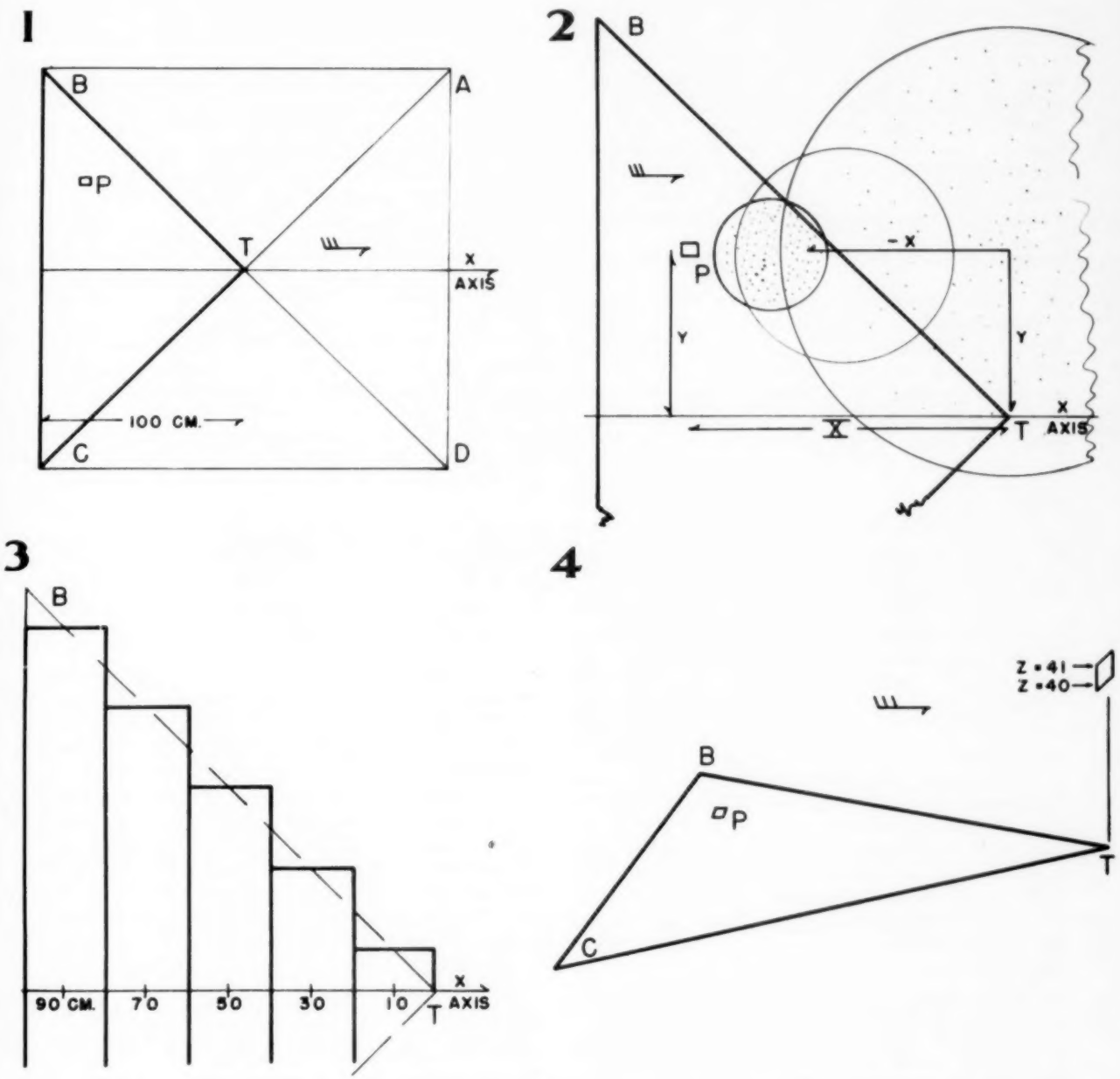

FIG. 1–4. FIG. 1. Plan view of spore trap (above point T) in a bed of diseased tobacco seedlings, 1956. Arrow flies with the wind.—FIG. 2. The enlargement of the outline of a cloud of spores from unit area P and the decrease in the concentration of spores in the air as the cloud moves downwind.—FIG. 3. The manner in which the numbers of air-borne spores are summed in the y direction at intervals of 20 cm in the X direction.—FIG. 4. The manner in which the numbers of air-borne spores are summed in the z direction from z = 40 to z = 41 cm.

$$S = \int_{0}^{100} \int_{40}^{41} \int_{-X}^{X} \int_{-\infty}^{\infty} s \, dx \, dy \, dz \, d X$$

where X is the horizontal distance downwind from P to T (Fig. 2).

The figures assist us in showing the reasons why the 4 summations give the number of spores, S, passing through the 1-cm² plane in a morning. First, since a long time is permitted for the cloud to travel downwind, the cloud will pass the trap at T from $x = -\infty$ to $x = +\infty$; therefore, the first summation is made along x from $-\infty$ to $+\infty$. Second, we must set limits on the summation along y. The horizontal distance, y, from the center of the cloud to the trap T as the cloud passes is equal to the distance from the source P to the X axis. Thus, we must sum from line BT to line CT or from $y = -X$ to $y = X$. Since the variance of the distribution of spore concentrations has been estimated to be $\frac{X^{7/4}}{2}$ (5), this summation can be made numerically from tables of the normal distribution. The summation was made at X = 10, 30, 50, 70, and 90 (Fig. 3). The third summation, along z, is only over the trap height, z = 40 to z = 41; this is performed by multiplying S at z = 40 and X = 10, etc. by 1 (Fig. 4). The final summation, along X, is made by multiplying each of the 20-cm increments employed (Fig. 3) by 20 and then adding them.

These considerations lead to

$$S \cong 4/10 \, q$$

But S has already been estimated to be 500 spores/cm²/day, thus

$$q \cong 10^3 \text{ spores/cm}^2\text{/ day.}$$

Recalling that 200 lesions were in the 4-m² area, we see that each lesion accounted for 200 cm² or produced about 2×10^5 spores/day.

Whether this estimate is realistic can be tested by considering a previously reported (7) outbreak of blue mold in shade tobacco in 1954. In 1954, 23 blue mold lesions produced sufficient spores in 1 day to cause 168 lesions on the 30 plants between 91 and 182 cm from the source. If we assign an area of 400 cm² to each plant, this corresponds to 0.014 lesion/cm² at a mean distance of 137 cm from the source. The lesions/cm², D, can be expressed in terms of the proportion of spores, p, trapped by the foliage; of the source strength Q_0; and of the infectivity, K, of the spores (5):

$$D = 0.135 \, p(Q_0/K)X^{-15/8} \, e^{-6.78 \, pX^{1/8}}.$$

The p is about 1/10 (2, 5); we have assigned $23 \times 2 \times 10^5$ spores/day to Q_0 above. Then $K \cong 100$ spores/lesion, a realistic estimate.

These observations in 1954 can be viewed in another way. The 4×10^6 spores disseminated by the source produced about 10^3 lesions in the 15×20-m field. That is, our calculations lead to the conclusion that 1 spore in 4×10^3 produced a lesion. Of the thousands that failed, 100 were deposited but failed, and thousands lit on the soil or were carried away by the wind.

Still another test of the plausibility of the estimates can be made by comparing them with spore production determined in the laboratory for a different, but closely related, fungus. Between 10^3 and 10^4 spores can be washed from a late blight lesion produced by *Phytophthora infestans* after a 12-hour sporulation period at 10°–22°C (J. R. Wallin, private communication). This is considerably less than the estimate of 2×10^5 spores per day per lesion for blue mold in the field. The comparison suggests that a large proportion of the spores removed by washing are also removed in the field. The 20- to 200-fold difference is not surprising when the differences in fungi and in method of removal and the assumptions necessary in our calculation are considered.

The time of dispersal is best shown by the results of 1955. In 1956, although the weather and size of outbreak were different from those of 1955, the same pattern can be seen. The cycle is as predicted by de Bary's and Pinckard's observations of the distortion of sporophores and the discharge of spores upon drying. The spores are rarely air-borne at night but fly in maximum numbers in the morning. The maximum occurs at 8–10 a.m. on cloudy days or at 1–5 a.m. on clear days. Dissemination is greater on cloudy days, when it may continue until 7 p.m., than it is on clear days.

The diurnal cycle of dissemination indicates that Peronospora spores are discharged as the sporophore shrivels in the morning but not while it is shriveled nor as it is wetted in the evening. The absence of dissemination at midday means that forcible discharge is necessary or that the spores on the structures lying on the leaf surface are not available to the turbulent air. The absence of air-borne spores in the evening indicates that the forcible discharge mechanism does not work when the sporophore is rewet and rises, and it also means that turbulence alone—without forcible discharge—cannot disseminate Peronospora spores.

The preceding conclusions are of fundamental importance in forecasting the disease and in controlling it with fungicides. For example, if a bright, hot day is predicted, we expect that spore dispersal will be light and early and that few spores will survive the day. Rain or dew that night will not arrive in time to save the spores. Therefore, increase of the disease is not forecast and dusting is of little value. If a bright day dawns, but rain is forecast by noon, a few spores will survive—particularly if the preceding day was rainy. In this case, some increase is forecast and morning dusting will be helpful. If a cloudy, cool day is forecast, maximum dissemination and infection is also forecast and immediate dusting is imperative. If the day is rainy and the leaves are continuously wet, slight dissemination will occur that day, but the next day will be especially dangerous because of the in-

creased dispersal of spores to leaves from which the fungicide has been washed.

In addition to dissemination per lesion and the daily cycle of spread, one would like to know about the weekly cycle and the effect of weather. The data of 1955 and 1956 suggest that these effects exist. In the first year (Table 1), dissemination decreased from the maximum on June 2, and by June 8 dissemination had increased again. These fragmentary data indicate a weekly cycle and/or a decrease due to sunny weather and an increase due to cloudiness.

A similar situation existed in 1956. The maximum concentration of air-borne spores occurred on day 6 and on days 13 and 14. These 2 periods followed rainfall and occurred at a 1-week interval. Thus, we can infer that weather and/or a weekly cycle influence the dissemination of spores of *P. tabacina*, but we cannot separate the 2 effects with the data at hand.

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LEAFROLL (WHITE EMPEROR DISEASE) OF GRAPES IN CALIFORNIA [1]

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SUMMARY

Fruit and foliage symptoms of grape leafroll indicate that it is identical with the virus disease known for several years in California as white Emperor disease. Fruits on affected vines generally have less sugar at harvest time than those on healthy vines of the same variety. The color of fruits on affected vines of varieties that normally produce red berries remains greenish white. Leaves on affected vines generally roll downward and chlorotic areas develop along the margins and between the principal veins by midsummer. The chlorotic areas turn red on leaves of varieties that produce red or black fruit. On leaves of many varieties, the chlorotic areas may become necrotic so that the foliage in the fall appears burned. Foliage symptoms appear first on the oldest leaves near the base of the canes. Symptoms of leafroll are similar to those of brunissure, rougeau, flavescence, and potassium deficiency. The use of virus-infected stocks or buds in propagating new vines appears to be the principal method by which the disease is spread in California.

Studies at the U. S. Horticultural Station at Fresno indicate that the disease characterized by white fruit on Emperor grapes and called white Emperor disease is the same as that described in 1936 by Scheu (11) as leafroll (Rollkrankheit). In keeping with priority and also because leafrolling is a more consistent symptom of the disease than is lack of fruit color, the name leafroll should be recognized and the name white Emperor should be dropped.

Leafroll is a serious virus disease of grape. Scheu (11, 12) estimated that over 80 per cent of all grapevines planted in Germany are infected; some varieties are so completely diseased that the varietal descriptions are based on leafroll-affected plants. He found the disease in native vines of *Vitis silvestris* Gmelin in Germany and stated that it had been present in German vineyards since olden times. He emphasized that infected plants are abnormally sensitive to environment and consequently are irregular bearers. During favorable seasons they may produce almost as well as healthy plants, but in unfavorable seasons they may produce only one fifth as well. The quality of the fruit, and therefore of the must produced from infected vines, is invariably poorer than that from healthy vines.

The purpose of this report is to bring together the descriptions of leafroll and to describe the sequential

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development of leafroll symptoms that will assist in diagnosis of the disease and in its elimination from American vineyards.

HISTORY OF LEAFROLL IN CALIFORNIA.—Olmo and Rizzi (7) called attention to a disorder of vines of the Emperor variety characterized by failure of the fruit to turn normal red at harvest time. They showed that progeny from such vines were likewise affected. Harmon and Snyder (3) called this disorder white Emperor disease and proved by graft-transmission tests that it was caused by a transmissible agent, i.e. a virus. Hewitt (6) recognized leafrolling, early autumnal coloration, and scorching as symptoms of white Emperor disease. Harmon (2) proved that the white Emperor virus could infect Cardinal and Red Malaga, in which it also prevented normal red coloration of fruit. In these varieties, as in Emperor, he found fruit on infected vines to be low in sugar. Coincident with this work it was noted that many vines in the varietal collection at the U. S. Horticultural Station, Fresno, showed leaf scorch, and records indicated that they had been so affected for several years. Harmon and Weinberger (4) selected inoculum from each of 32 varieties from this collection that had shown leaf scorch and obtained transmission to Emperor, with the production of characteristic white fruit, from each of them.

Observations in commercial vineyards in the vicinity of Fresno and elsewhere in California indicate that leafroll-like symptoms are very prevalent and that this disease may be the cause of severe economic losses to growers. In some varieties in which the disease does not alter fruit color, it has been present for a long time but not recognized as a disease. Cuttings and graft wood from such infected plants may have served for inadvertent spread of the disease. In preliminary surveys, leafroll-like symptoms were found to be more prevalent in vineyards of varieties on rootstocks than in those of varieties on their own roots, indicating that use of infected rootstocks has been a major factor in spreading the disease.

The apparent wide occurrence of the disease in many varieties and its almost universal presence in some varieties agree with Scheu's report of the disease occurrence in Germany (11, 12) and tend to indicate that leafroll virus was introduced into California in infected vines from Europe.

SYMPTOMATOLOGY.—During the 1956 growing season, the development of symptoms in infected vines was watched closely at Fresno, California. From the time that growth started in the spring until the latter part of July, no outstanding differences of growth of canes and development of leaves and flower clusters on infected plants and on adjacent plants known to be free of the disease were recorded.

By the end of July, symptoms on infected plants had developed sufficiently to distinguish such plants from healthy ones. The earliest symptom observed was failure of normal fruit color to develop in Cardinal; this was noted by July 25, 1956. Leaf symptoms on most varieties were observed initially during the first week in August.

Fruit color at harvest time proved to be diagnostic for distinguishing leafroll in red-fruited varieties but was of little or no value for detecting the disease in black- or white-fruited ones. Color differences in fruit of healthy and of diseased vines of red vareties appeared as soon as the fruit started to color and were maintained throughout the season. The fruit from infected vines of black-fruited varieties turned as black as did that on healthy vines. The fruit-color differences on white-fruited varieties were very slight.

Comparative amounts of sugar in the fruit also proved to be a good character for diagnosing leafroll in some grape varieties. Measurements by refractometer showed that the sugar in individual berries of infected vines was generally only about ½ to ⅔ that of berries from noninfected vines. The sugar from berries in marked clusters of Cardinal was determined at weekly intervals, starting in mid-August. The fruit from healthy vines finally attained a sugar content of about 25 per cent; that from infected vines never exceeded 16 per cent. Similar differences in sugar content between the fruit of infected and noninfected vines of the varieties Blackrose, Calmeria, and Emperor were found. The sugar content of fruit of many varieties for which no healthy vines were on hand for comparison was less than 18 per cent when measured on October 12, 1956. The sugar content of fruit from infected vines of other varieties, however, reached acceptable levels by this date, when the percentage of sugar was 19 in Palomino; 20 in Semillon; 21 in Boal de Madeira; 23 in Pinot noir, Roussane, and Sauvignon vert; and 26 in Refosco. Fruit on vines of Muscat of Alexandria grafted to roots of infected Emperor contained 29 per cent sugar on September 19. This was approximately the same as the sugar content of nearby healthy vines of that variety.

Distinctive downward roll of the leaf margins (Fig. 1) and leaf chlorosis were very constant characteristics of infected vines of most varieties. Rolling showed first on the oldest leaves near the base of the canes during the first week in August. The rolling became more pronounced as the season progressed; in September and October, most of the mature leaves on the infected vines showed the downward rolling, but the small leaves near the tip of the canes remained green and more or less normal. Chlorosis of the margins and of the intercostal parenchyma of leaves appeared coincidentally with symptoms of rolling. The principal veins and a small band along each remained green. Later, the leaves became brittle and rugose and had sunken veins and a rough somewhat glossy appearance of the intercostal areas. The chlorotic areas turned red on red- and black-fruited varieties, but they remained yellow on white-fruited varieties. On many varieties, brown necrotic spots developed in the middle of the chlorotic areas. These spots enlarged and coalesced until the entire leaf blade, except at the base of the principal veins, was necrotic

and looked burned. On some varieties, the surface of the necrotic leaves developed a silver color.

Vines affected with leafroll do not always show burned-foliage symptoms. In their original report, Harmon and Weinberger (4) noted that no burned-foliage symptoms developed on the index Emperor vines during the first year. There were, likewise, no burned-foliage symptoms during the second year, although the characteristic symptoms of rolling, rugosity, and chlorosis developed on the index vines but not on the nonbudded check vines. Fruit on the diseased index plants was again greenish white and low in sugar.

In the 1956 season, a shoot of the American rootstock 1613 growing from the base of an infected vine developed no characteristic symptoms of leafroll, but a vinifera top variety (Cardinal) showed typical fruit and foliage symptoms.

LEAFROLL, WHITE EMPEROR DISEASE, AND OTHER GRAPE DISORDERS.—Comparison of the foliage and fruit symptoms of leafroll as described in Europe (11) with the disease previously called white Emperor in California clearly shows that they are the same. Symptoms of both show first in late July or early August, when the fruit is starting to mature. Leaf symptoms of both develop initially on the oldest, lowest leaves of the canes. Rolling of the leaf margins toward the back of the lamina is characteristic of both. Premature coloring of the leaves of infected plants is likewise characteristic of both. Both cause poor fruit quality and low fruit-sugar content. In addition, both are systemic diseases caused by agents transmissible to all progeny vines. Also, both affect vinifera grapes severely but have very little or no effect on American rootstocks.

FIG. 1. Healthy leaf (left), and top (center) and bottom views (right) of leafroll-affected Mission grape leaves from indexing plots at Davis, California, November, 1956.

On the basis of symptomatology, the grape diseases brunissure, rougeau, and flavescence described in the French literature (8, 10) are also very similar to leafroll. In the original description of brunissure, Pastre (8) noted that leaf symptoms appeared first from August 1 to 15; the leaves colored prematurely and burned late in the season. At harvest, the fruit did not mature but remained uncolored and low in sugar. In their description of rougeau and flavescence, Ravaz and Verge (10) likewise noted that these diseases were apparent only during the harvest season. The leaves of affected plants were rolled towards the back of the lamina, they colored prematurely, and they were rugose and thickened. Fruit maturity was adversely affected. Varieties with red or black fruit were affected by rougeau and those with white fruit by flavescence, otherwise the diseases were identical.

After observing leafroll in Germany, Branas (1) and Stellwaag and Branas (13) recognized the similarity between it and rougeau-flavescence. They speculated that leafroll might be related to court-noué and that it might be a form of flavescence caused by a transmissible agent; however, transmission of the causal agent of rougeau-flavescence apparently has not been reported from France.

There are no similarities between leafroll and the disease (9) called arricciamento by the Italian workers; *arricciamento* is sometimes translated into English as *leafroll* or *leaf curl*.

Conditions other than virus infection may cause leafroll-like symptoms in grape plants. Scheu (11, 12) listed mechanical bending, Botrytis infection, plough wounds, and nutritional disorders as possible causes of such symptoms. Scheu corrected all of these by care or with proper amendments and found that no leafroll virus could be transmitted from plants with such symptoms. Similarities between leafroll and some nutritional disorders have resulted in confusion and may have partially caused the long delay in the recognition of leafroll as a distinct virus disease.

Symptoms of leafroll and those of potassium deficiency in particular are very similar. Herschler (5) found in many German vineyards scattered vines that showed symptoms like those of potassium deficiency during the autumn and that had a very low level of potassium in the leaves. The soil in which these plants were growing, however, was well supplied with potassium, and the adjacent vines showed no potassium-deficiency symptoms. Since leafroll and potassium deficiency both produce symptoms of leaf rolling and marginal burning, and since Scheu (11) found a high incidence of leafroll in German vineyards, there is little doubt that Herschler was observing leafroll-affected vines. Wilhelm (14) also noted the similarity of brunissure as described by the French authors, the potassium-deficiency-like symptoms described by Herschler (5), and known potassium deficiency. In particular, he recognized the close similarity of symp-

toms in the final stages of necrosis and of the effects of growth and yields. He suggested that further research was urgently needed to obtain a more exact understanding of potassium deficiency in the vine. To this we wholeheartedly agree and suggest that an effort be made to distinguish clearly virus-induced leafroll from true potassium deficiency.

LEAFROLL CONTROL.—Leafroll might well be one of the most important problems facing viticulture today. When the importance of the problem is recognized, however, control should not be difficult. Scheu (12) found no soil retention of the virus, and he did not find a vector. Although there may be some natural spread in German vineyards, indications are that there is very little if any natural spread in California. The removal of infected vines and the use of vines free from the disease for further plantings should bring about effective control.

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A QUALITATIVE COMPARISON OF THE AMINO ACID AND SUGAR CONTENT OF ACID HYDROLYSATES FROM THE MYCELIUM OF SEVERAL ANTHRACNOSE FUNGI [1]

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SUMMARY

The amino acid and sugar composition of the mycelium of *Colletotrichum tabacum* Averna-Sacca, *C. lagenarium* (Pass.) Ell. et Halst. Races I and II Goode, *C. capsici* (Syd.) Butler et Bisby, and *Gleosporium piperatum* Ell. et Ev. was studied by qualitative paper chromatography. Both sugars and amino acids were determined from acid hydrolysates prepared from mycelial cultures grown in a minimal mineral medium in shake culture. Potassium nitrate and glucose were the only sources of nitrogen and carbon provided for fungal growth. Fourteen amino acids and 5 sugars were identified from the hydrolysates. Qualitative differences existed among species and strains of these fungi with respect to specific amino acids and sugars.

The use of paper chromatography as a research tool to aid in an understanding of some of the basic differences among strains, species, genera, or other groupings of the fungi has received little attention. Broyles (2) found qualitative and quantitative differences in the amino acids and sugars present in the urediospores of certain races of cereal rust fungi. Other workers have studied specific organisms from the standpoints of their nutritional requirements and of their subsequent amino acid composition (4, 6, 9, 10).

Taxonomists in other fields have turned to chromatography as a taxonomic tool (1, 3). Micks (8) found that 3 genera of mosquitoes could be distinguished readily by marked quantitative differences in their amino acid levels. Similarly, Ginsburg and Lovett (5) distinguished between 2 strains of a bacterium, one strain having a glutamic acid content much greater than that of the other.

The present investigation was undertaken to determine if detectable qualitative differences in amino acid and sugar content might exist among closely related fungi. The organisms selected were *Colletotrichum tabacum* Averna-Sacca; *C. lagenarium* (Pass.) Ell. et Halst. (Races I and II Goode); *C. capsici* (Syd.) Butler et Bisby; and *Gleosporium piperatum* Ell. et Ev.

MATERIALS AND METHODS.—Mycelium of the organisms was transferred from potato-dextrose agar slants to flasks containing 35 ml of a liquid medium composed of potassium nitrate (10.0 g), potassium dihydrogen phosphate (5.0 g), magnesium sulfate (2.5 g), glucose (20 g), and water (1000 ml). The flasks were held on a shake apparatus for 7 days at room temperature. *Colletotrichum capsici* and *Gleosporium piperatum* grew well, *C. tabacum* made moderate growth, and *C. lagenarium* made limited growth during this period.

The mycelium of each of the 5 fungi was separated from the medium by centrifugation and any adhering material removed by washing with water. The mycelium was dried at 40° C, and a weighed portion was then hydrolyzed for 8 hours at 85° C with 2 N H_2SO_4. The resulting hydrolysate was adjusted to pH 6.5 with $CaCO_3$, and the supernatant was removed by centrifugation. The slurry of $CaCO_3$ was washed with 10 ml of 50 per cent ethyl alcohol, and the washings were recombined with the supernatant.

In the separation of the sugars, the supernatant was dried *in vacuo* at 40° C and the salts washed twice with 10 ml of pyridine. The pyridine was filtered and then evaporated at 35° C. The sugars in the resultant residue were dissolved in 1 ml of 50 per cent aqueous isopropyl alcohol. From the alcohol solution the sugars were spotted on Whatman No. 1 filter paper and identified by the procedure of Lynch, Wright, and Olney (7); the solvent was a mixture of butyl alcohol, acetic acid, and water in a ratio of 4:1:5. The sugars were identified by spraying with Partridge's reagent and drying at 105° C in an oven.

For the preparation of the amino acids, nearly neutral supernatant, obtained after hydrolysis of the mycelium, was reduced to several ml by evaporation *in vacuo* and by freezing followed by filtering. The procedure was repeated until the salt residue was almost completely removed. The small volume of liquid was then spotted on Whatman No. 1 filter paper and placed either in a solvent of phenol and water (80:20) or in one of butyl alcohol, acetic acid, and water (4:1:5). This method has been found to give results comparable to the removal of the amino acids by cation-exchange resins. The solvent was allowed to advance about 6 in. up the paper; the paper was then removed, air dried, and replaced in the solvent so that the wetting front advanced to about 6 in. above the former front. This procedure was repeated 3 times; the paper was then removed, air dried, and sprayed with a 2 per cent solution of ninhydrin in butyl alcohol. The unknowns were then identified by comparison of their R_f values with the R_f values of known amino acids.

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TABLE 1.—*The relative amino acid contents of the mycelia of 5 anthracnose fungi*

Amino acid	Relative content[a] in indicated fungus[b]				
	C.t.	C.c.	C.l.-I	C.l.-II	G.p.
Lysine	+	+	+	+	+
Arginine	—	+	+	+++	+
Glutamic acid	—	+++	++	++	—
Serine	—	+	++	++	—
Valine	+	+	+	++	+
Aspartic acid	+	+	+	+	+
Leucine	+	+	++	+	+
γ-Butyric amino acid	—	++	+	+	+
Glycine	+	—	—	+++	—
α-Alanine	—	+	+	+	—
Threonine	—	+	+	+	+
Unknown 1	+(red)	—	—	—	—
Unknown 2	—	+ (red)	—	—	++(red)
Unknown 3	—	—	—	+ (red)	—

[a] Where — indicates no color and +, ++, and +++ indicate relative intensities of color.
[b] C.t.—*Colletotrichum tabacum*; C.c.—*C. capsici*; C.l.-I—*C. lagenarium* (Race I); C.l.-II—*C. lagenarium* (Race II); and G.p.—*Gleosporium piperatum*.

RESULTS.—Qualitative differences in amino acids and sugars were found among the 5 fungi (Tables 1, 2). Of the 14 amino acids found, 11 were identified as lysine, arginine, glutamic acid, serine, valine, aspartic acid, leucine, γ-amino butyric acid, glycine, α-alanine, and threonine; 3 unidentified materials that gave a pink to red color with ninhydrin also were present. Of the known amino acids, lysine, valine, aspartic acid, and leucine were common to all 5 fungi. The number of identified and unidentified amino acids for each organism was as follows: *Colletotrichum tabacum*, 5 identified, 1 unidentified; *Gleosporium piperatum*, 7 identified, 1 unidentified; *C. capsici*, 10 identified, 1 unidentified; *C. lagenarium* (Race I) 10 identified, 0 unidentified; and *C. lagenarium* (Race II), 11 identified, 1 unidentified.

C. capsici and *G. piperatum*, which cause similar disease symptoms on pepper fruit but have different spore sizes and shapes, differed under the conditions of this test in that *C. capsici* contained glutamic acid, serine, and alanine whereas *G. piperatum* did not.

Races I and II of *C. lagenarium*, which differ in that Race II will attack varieties of watermelon that Race I will not, differed with respect to their amino acid content only in that Race II had a high content of glutamic acid whereas Race I contained none. The hydrolysate of Race II contained 1 unidentified amino acid whereas all amino acids in the hydrolysate of Race I were identified.

C. tabacum contained the least number of identified amino acids. It did not contain γ-butyric amino acid or threonine, which were common to the other 4 fungal cultures.

Qualitative differences also existed with respect to the presence or absence of sugars in the mycelium of the organisms. All of the isolates contained glucose, xylose, mannose, and 2.3.4.6-tetramethylglucose. *C. tabacum* and Race I of *C. lagenarium* did not contain galactose whereas *C. capsici*, *G. piperatum*, and Race II of *C. lagenarium* did.

DISCUSSION.—The data indicate that the 5 fungi synthesize a number of amino acids when supplied with a simple inorganic nitrogen source and form a number of sugars when provided with a single simple sugar. It is probable that a greater number of amino acids and sugars would be formed if organic sources of nitrogen were provided.

The fact that qualitative differences in amino acid and sugar content exist among species within the same genus suggests that such differences may be helpful in separating or grouping strains, species, or genera of doubtful position. It is also possible that a study of amino acid or sugar differences, and the ability to synthesize them from a given substrate, will aid in a better understanding of the physiology of parasitism. Caution must be exercised, however, in the interpretation of chromatographic patterns. The extent of variability within a species could be as great as that existing between species and would, necessarily, have to be determined. It is possible that the presence of

TABLE 2.—*Presence or absence of various sugars in the acid hydrolysates of 5 anthracnose fungi*

Sugars	Relative contents[a] in indicated fungus[b]				
	C.t.	C.c.	C.l.-I	C.l.-II	G.p.
Glucose	+	+	+	+	+
Galactose	—	+	—	+	+
Xylose	+	+	+	+	+
Mannose	+	+	+	+	+
2.3.4.6-Tetramethylglucose	+	+	+	+	+

[a] Where — indicates no color and +, ++, and +++ indicate relative intensity of color.
[b] C.t.—*Colletotrichum tabacum*; C.c.—*C. capsici*; C.l.-I—*C. lagenarium* (Race I); C.l.-II—*C. lagenarium* (Race II); and G.p.—*Gleosporium piperatum*.

glycine and of galactose in Race II of *C. lagenarium* and their absence in Race I may be of significance, but such a broad generalization cannot be drawn until this species and its races from many areas are sampled. Similarly, the differences that exist among fungus species may be useful in a taxonomic study, subject to adequate sampling and control of the experimental environment. The use of paper chromatography as a research tool in this respect should be further investigated.

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THE EFFECT OF WATER AND POTASSIUM PHOSPHATE BUFFER ON PLANT VIRUS INFECTION PRIOR TO INOCULATION[1]

Robert P. Kahn[2] and John L. Libby

SUMMARY

The preinoculation effects of the presence of water and of 0.1M potassium phosphate buffer (pH 7) on the infection of primary leaves of Pinto beans by tobacco-mosaic virus and southern bean-mosaic virus was determined by comparing relative numbers of local lesions. An interaction was demonstrated between groups of leaves subsequently inoculated with a phosphate-diluted inoculum and comparable groups inoculated with a water-diluted inoculum.

When the inocula were diluted with water, leaves that received a preinoculation abrasion in the presence of phosphate buffer and/or dry abrasion developed significantly more lesions than did leaves that were abraded in the presence of water. Leaves that received dry abrasion and then a dip in phosphate buffer, or no dip, preceding inoculation developed more lesions than did leaves that received dry abrasion and a dip in water prior to inoculation.

When the inocula were diluted with phosphate buffer, leaves that received dry abrasion developed more lesions than did leaves receiving abrasion in the presence of water or of phosphate buffer. Similarly, lesion counts were higher on leaves that received no dip between dry abrasion and inoculation than on leaves that were dipped into water or phosphate buffer prior to inoculation.

Leaves remained receptive to infection at least 80 minutes after they were abraded. The duration of the receptive period apparently was not affected by either the type of abrasion or the presence or absence of added phosphate in the inoculum.

There were significantly more infections on leaves that received phosphate-diluted inocula than on leaves that received comparable water-diluted inocula, regardless of the preinoculation treatment, thus confirming, on the basis of a comparatively larger leaf population, the previously reported finding that the presence of potassium phosphate in the inoculum is associated with higher lesion counts.

The stimulatory action of potassium phospate on plant virus infection was first reported by Thornberry (7) and subsequently by other investigators as reviewed by Yarwood (11). An inhibitory effect of water on plant virus infection also has been reported. For example, Yarwood (9) reported that more tobacco mosaic virus lesions developed on Carborundum-dusted leaves of *Nicotiana glutinosa* L. and of bean

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TABLE 1.—*Number of local lesions on Pinto Bean leaves abraded by 3 methods and then dipped into an inoculum diluted with distilled water or with 0.1M potassium phosphate buffer at pH 7*

Diluent of inoculum	Method by which Celite-dusted leaves were abraded	Mean no. of local lesions/leaf [a] Tobacco-mosaic virus 1955	Tobacco-mosaic virus 1956	Southern bean-mosaic virus 1955	Southern bean-mosaic virus 1956
Water	Dry spatula	70	60	69	78
	Spatula dipped in water	74	24	79	42
	Spatula dipped in phosphate	156	58	165	106
Phosphate	Dry spatula	393	330	275	292
	Spatula dipped in water	349	200	248	175
	Spatula dipped in phosphate	311	118	272	156
LSD 5%		66	72	30	86
Means for diluents					
Water		100	47	104	75
Phosphate		351	216	265	208
LSD 5%		66	39	34	112

[a] In 1955, 6 trials of 17–20 leaves each were made; in 1956, there were 6 trials, each with 10 leaves.

that were abraded with a dry brush than on leaves abraded with a wet brush prior to inoculation with a K_2HPO_4-diluted inoculum. Yarwood (8) reported similar results also when the inoculum contained no added phosphate. Allington and Laird (1) observed fewer tobacco-mosaic lesions on dry, Carborundum-abraded leaves of *N. glutinosa* that were dipped into water prior to inoculation with a water-diluted inoculum than on comparable leaves that received no preinoculation water dip. Many of the data that have been reported appear to have been based on leaf samples too small to compensate for the high degree of variation caused by genetic and environmental differences in the susceptibility of the test plants.

Apparently no investigations have been reported in the literature concerning the preinoculation effects of water and of phosphate on plant virus infection where the water and phosphate variable were evaluated at the same time by relative comparisons with a standard or dry-treatment control. It was the purpose of the investigations reported herein to compare preinoculation water, phosphate, and dry treatments on comparatively large populations of test plants. It was also the objective of these studies to determine whether there was any interaction between any of these 3 types of preinoculation treatments and the presence (or absence) of added potassium phosphate in the inoculum. Some of the data have been reported previously in a preliminary form (3).

MATERIALS AND METHODS.—The viruses employed in these investigations were 1) tobacco-mosaic virus (TMV), *Marmor tabaci* Holmes, represented by a single-lesion isolate of the James Johnson strain supplied by H. H. Thornberry; and 2) southern bean-mosaic virus 1 (SBMV) = bean mosaic virus 4, *Marmor laesiofaciens* Zaumeyer & Harter supplied by W. J. Zaumeyer. These cultures were propagated in tobacco (*Nicotiana tabacum* L. var. Turkish or Havana 38) or in beans (*Phaseolus vulgaris* L. var. Commoner or Brittle wax). Leaves on inoculated plants showing

TABLE 2.—*Number of local lesions on Celite-dusted Pinto bean leaves that were rubbed with a dry spatula and then either were or were not dipped into water or phosphate buffer prior to dipping in an inoculum diluted with water or with 0.1M phosphate buffer at pH 7*

Diluent of inoculum	Types of dip between dry abrasion and inoculation	Mean no. of local lesions/leaf [a] Tobacco-mosaic virus 1955	Tobacco-mosaic virus 1956	Southern bean-mosaic virus 1955	Southern bean-mosaic virus 1956
Water	No dip	170	108	213	64
	Water dip	82	22	121	34
	Phosphate dip	147	112	221	92
Phosphate	No dip	379	546	318	268
	Water dip	191	174	283	106
	Phosphate dip	184	160	224	110
LSD 5%		48	75	41	94
Means for diluents					
Water		133	81	185	95
Phosphate		251	293	275	161
LSD 5%		44	28	42	58

[a] In 1955, there were 6 trials of 17–20 leaves each; in 1956, there were 5 trials of 10 leaves each for TMV and 3 trials of 10 leaves for SBMV.

typical symptoms were harvested, shredded, and dried over $CaCl_2$ at 4°C according to the method of McKinney (4) and stored over Anhydrone (magnesium perchlorate) in sealed glass jars at 4°C until utilized for inoculum preparation. Inoculum was prepared by extracting finely ground dry tissue with distilled water and diluting portions with equal volumes of distilled water or 0.1M potassium phosphate buffer at pH 7. The weight of desiccated tissue and the volume of dilutions were predetermined for each lot of tissue so as to yield a countable number of local lesions on test plants.

Beans (*Phaseolus vulgaris* var. Pinto) were used in all the local-lesion assays. Plants were propagated in Duffield silt loam in ¼-gal. glazed pots. Plants with opposite primary leaves of uniform size and area were selected from large plantings. The leaves were inoculated 8 days after planting during summer months and 10 days after planting during winter months.

Unless specified otherwise later in the report, primary leaves that had been subjected to various preinoculation treatments were detached and then inoculated by dipping the leaves either into an inoculum diluted with water or into one diluted with 0.1M potassium phosphate buffer (pH 7). Leaves were immersed in the inoculum 3 times in quick succession. The excess inoculum was drained on paper towels for 10–15 seconds.

The inoculated, detached leaves were incubated in 20 × 150-mm Petri dish moist chambers containing wet filter paper. Incubation was carried out under continuous fluorescent light (day-light type, 200–300 foot-candles) to delay the yellowing of leaves beyond the 4- to 5-day incubation period. At the end of the incubation period, the lesions were counted under slight magnification and illumination afforded by a Quebec colony counter. The mean lesion counts derived from a group of leaves that received the same treatments were subjected to an analysis of variance in a split-plot design. The various preinoculation treatments were compared in the main plots (pairs of opposite primary leaves) and inocula types in the subplots (1 leaf of each pair).

RESULTS. — *Methods of abrasion.* — Celite-dusted leaves were abraded 1) with a dry ground-glass spatula, 2) with a spatula dipped in water before each stroke, or 3) with a spatula dipped in 0.1M potassium phosphate buffer (pH 7). After abrasion, leaves were inoculated by dipping them into an inoculum diluted with either water or phosphate buffer.

The relative number of local lesions was influenced both by the nature of the abrasive treatment and the type of inoculum (Table 1). Lesion counts were significantly higher when leaves abraded by any given method were dipped in an inoculum diluted with phosphate than when dipped in one diluted with water. In the 1955 tests, with inocula diluted with water, abrasion in the presence of potassium phosphate resulted in significantly more lesions than did dry abrasion or abrasion in the presence of water. The 1956 results differed in that more lesions occurred on leaves that received abrasion in the presence of phosphate, or dry abrasion, than on leaves abraded in the presence of water. With inocula containing phosphate, leaves that received dry abrasion developed more lesions than did leaves abraded in the presence of water or phosphate. Of the several combinations tested, that of dry abrasion and use of inoculum containing added phosphate resulted in the highest number of lesions. The phosphate effect was greater when phospate was added to the inoculum than when applied at time of abrasion.

Dipping between abrasion and inoculation.—Lesion counts on Celite-dusted leaves that received dry abrasion and a dip in either water or phosphate buffer prior to inoculation were compared to counts obtained from leaves that received dry abrasion but no preinoculation

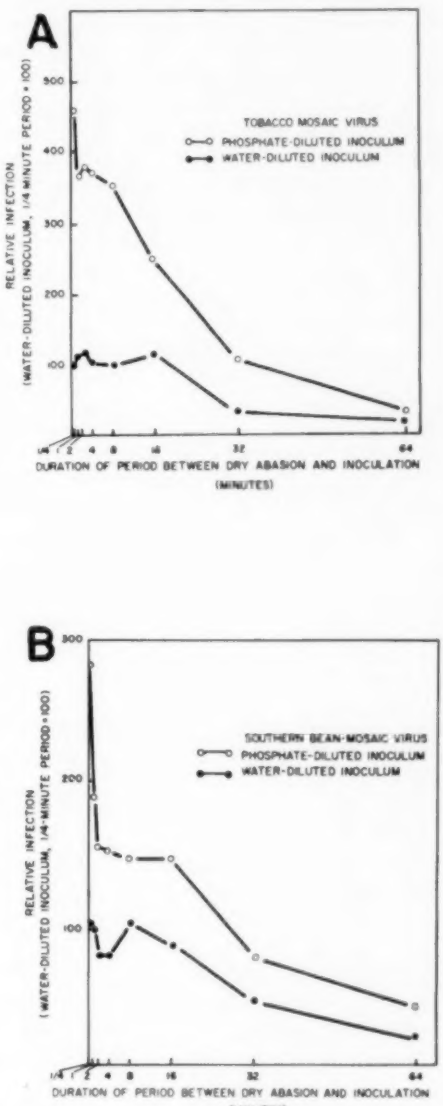

FIG. 1. Relative infection on primary leaves of Pinto beans as influenced by the duration of the receptive period between dry abrasion and inoculation. A) Tobacco-mosaic virus. B) Southern bean-mosaic virus.

dipping treatment. Local lesion numbers were influenced by both the dipping treatment and the type of inoculum (Table 2). There were more lesions on leaves inoculated with an inoculum diluted with phosphate buffer than on leaves inoculated with a water-diluted inoculum. With inocula diluted with water, lesion counts for leaves that received a preinoculation dip in phosphate were approximately the same as for leaves that received no preinoculation dip, but both were much higher than on leaves receiving a water dip. With inocula diluted with phosphate, lesion counts were much higher on leaves that received no preinoculation dip than on leaves that received either a water or phosphate dip.

Duration of the receptive period. — Celite-dusted leaves were abraded with a dry ground-glass spatula 320, 160, 80, 40, 20, 10, 5, or ¼ minutes prior to inoculation. Then all leaves were sprayed with an inoculum diluted either with water or phosphate buffer. Lesion count means for 5 trials of 7–12 leaves for each time period were progressively lower as the time between abrasion and inoculation increased. The bean leaves remained receptive to infection by TMV or SBMV at least 80 minutes after dry abrasion for inoculations with water- or phosphate-diluted inocula.

In another experimental series, leaves received dry abrasion 64, 32, 16, 8, 4, 2, 1, or ¼ minutes before they were detached and dipped into an inoculum diluted either with water or phosphate buffer (Fig. 1). Initially there were 3–5 times as many lesions on leaves that were inoculated with phosphate-diluted inocula than on leaves receiving water-diluted inocula. As the time interval between abrasion and inoculation was increased, the ratio decreased so that at the end of the test period, lesion counts were approximately the same whether or not phosphate was added to the inoculum. Thus, the receptivity of abraded leaves to infection decreased as the time between abrasion and inoculation increased, but the rate of decrease was much greater for leaves that received a phosphate-diluted inoculum then for those that did not. Thus whereas phosphate has been shown in previously cited papers and in this paper to be associated with increased infection, these particular data suggest that phosphate in the inoculum may have an adverse effect if there is a delay between abrasion and inoculation. It is interesting to note that Jedlinski (2) observed with TMV in water-diluted inocula that lesion counts on bean leaves were approximately the same for delay periods of 0, 30, 60, 300, or 600 seconds whereas lesion counts were maximal at 300 seconds for other hosts.

In still another experimental series, intact Celite-dusted leaves received dry abrasion or abrasion in the presence of water or phosphate buffer ¼, 5, 10, 20 and 40 minutes prior to the spraying of an inoculum diluted with water or with phosphate buffer. Lesion counts were progressively lower as the duration of the period between abrasion and inoculation was increased. At the end of the 40-minute test period, lesion counts were approximately the same regardless of the preinoculation abrasion treatment. Thus the receptivity of abraded leaves was not altered by the type of abrasive treatment or by the presence or absence of added phosphate in the inoculum.

Discussion.—The phosphate effect reported herein for preinoculation treatment effects and for comparisons of water-diluted inocula and phosphate-diluted inocula was demonstrated by a 3-fold difference between lesion count means. In comparison, a 2-fold difference was reported by Takahashi (6) for TMV on tobacco and a 4- to 100-fold difference reported by Yarwood (9) for TMV on several bean varieties. Yarwood also demonstrated a smaller effect with 9 viruses on various hosts including tobacco. Yarwood and Takahashi suggested that tobacco is much less susceptible than beans to the action of phosphate.

The difference in magnitude in these various reports may be attributed also to a pH effect. Yarwood compared K_2HPO_4 at pH 8.5 with water presumably at pH 6, whereas Takahashi and the authors compared potassium phosphate buffer at pH 7.0 with water at approximately pH 6. Data concerning the effect of pH on infectivity as reported by Stanley (5) and Thornberry (7) suggest that a much higher difference in lesion counts might be expected for comparisons of pH 8.5 and pH 6 than for comparisons of pH 7 and pH 6.

The present data obtained from a comparatively large leaf sampling confirm the previously reported findings of several earlier investigators (11) that the presence of potassium phosphate in the inoculum results in more numerous lesions.

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